REFLEX RELEASE OF ADRENALINE AND NORADRENALINE FROM THE CANINE ADRENAL MEDULLA

BY

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Declaration

This thesis is entirely my own composition and the work detailed within is my own except where indicated.

SIGNED

ABSTRACT

The release of catecholamines from the canine adrenal medulla in response to various stimuli was investigated 'in vivo' and 'in vitro' to attempt to answer the following questions:-

1. Are the catecholamines adrenaline and noradrenaline selectively released by the canine adrenal medulla?

2. Are the baroreceptor and chemoreceptor reflex responses mediated by nicotinic and muscarinic receptors respectively?

3. Is there a humoral mechanism involving the anterior pituitary-adrenocortical axis controlling catecholamine release?

4. Are prostaglandins involved in maintaining adrenal blood flow?

The results in this thesis show:-

1. There is no evidence for selective release of adrenaline or noradrenaline during either chemoreceptor stimulation, baroreceptor reflex stimulation or corticotrophin infusion. However, the nicotinic antagonist, hexamethonium bromide, produced small but consistent increases in the resting percentage of noradrenaline relative to adrenaline. This observation is consistent with the findings of other workers.

2. The adrenomedullary response to baroreceptor and chemoreceptor reflex stimulation is significantly reduced by hexamethonium and, to a lesser extent, by hyoscine methyl bromide. This suggests that transmission of both the baroreceptor and chemoreceptor reflex to the adrenal medulla of the dog consists predominently of nicotinic receptor activation with a large subsidiary muscarinic component. Although the distinction between baroreceptor and chemoreceptor reflex transmission was poor there was some evidence to suggest selectivity of the baroreceptor reflex for nicotinic receptors and the chemoreceptor reflex for muscarinic receptors.

3. There are two components to the response of the adrenal medulla to carotid body hypoxia. The first is a rapid release of catecholamines which requires an intact nerve supply to the adrenal gland but is independent of adrenocortical function. The second is a delayed release of catecholamines which, in contrast to the rapid release, requires an intact pituitary-adrenocortical axis, but is independent of the motor nerves to the gland. Release of catecholamines from the canine adrenal medulla, in response to carotid body hypoxia, is therefore controlled by both neural and humoral mechanisms.

4. Adrenal blood flow is reduced both at rest and during reflex stimulation of the adrenal gland by the prostaglandin synthetase inhibitor, indomethacin. On comparing the resting pressure-flow curves of a range of dogs I found indomethacin significantly depressed the curve; therefore indicating prostaglandins do play a role in maintaining adrenal blood flow. I would further suggest prostaglandins are released with catecholamines and maintain

adrenal blood flow by opposing their constricting action. This may also explain why platelet aggregation due to high concentrations of catecholamines does not occur in adrenal veins.

CONTENTS

Introduction

Outline of Research

The first aspect of catecholamine release from the canine adrenal medulla we investigated was whether the two catecholamines, adrenaline and noradrenaline, could be released independently by carotid baroreceptor and chemoreceptor reflexes, with the dog under either pentobarbitone or chloralose-urethane anaesthesia.

This study was initiated from the results of earlier work in our laboratory, by Critchley and Ungar (1974). They tried to answer a similar question to the one above but compared catecholamine release in response to baroreceptor and chemoreceptor reflex stimulation in the cat under pentobarbitone anaesthesia with the dog under chloralose-urethane anaesthesia. Their results clearly demonstrated selective release of the two catecholamines in the cat; the baroreceptor reflex selectively releasing noradrenaline and the chemoreceptor reflex selectively releasing adrenaline. In the dog they found no evidence of selective release of either catecholamine in response to either reflex.

As it is known that anaesthetics can have a marked effect on catecholamine release from the adrenal medulla (see 'Methods - anaesthesia' and De Schaepdryver (1959)) we did not consider this study an ideal comparison of dog with cat. In order to ascertain if Critchley and Ungar's study reflected a true difference between dog and cat we repeated the study comparing dogs under both types of anaesthesia.

Whilst our study, in conjunction with that of Critchley and Ungar, only provides data comparing dog and cat, we hoped that it might close the long standing debate of whether adrenaline and noradrenaline are released independently. On reviewing the literature, we find most evidence in favour of selective release to come from studies on the cat (von Euler and Folkow (1953), Folkow and von Euler (1954), Duner (1953)) and most evidence against to come from studies on the dog (Malmejac (1964)). This would suggest a species difference as the simplest answer to the debate.

There is some evidence suggesting selective release in the dog, but in all cases the change in the noradrenaline:adrenaline ratio is small. Work done using muscarinic and nicotinic agonists and antagonists has revealed the following:-

Rapela and Houssay (1952)

Nicotine selectively releases adrenaline relative to noradrenaline.

Kayaalp and McIsaac (1968)

Methacholine selectively releases noradrenaline relative to adrenaline.

De Schaepdryver (1959)

Acetylcholine, in the presence of atropine, selectively releases adrenaline relative to noradrenaline.

This would imply the release of noradrenaline and adrenaline may be mediated by muscarinic and nicotinic receptors respectively, but as the changes observed are all small neither catecholamine can be under the sole control of any one mechanism. The main evidence against selective release by muscarinic and nicotinic agonists comes from our own laboratory (Critchley (1976)). However, all the evidence in favour of selective release comes from '*in vivo*' studies in the dog whereas Critchley's evidence against selective release is based on '*in vitro*' studies in isolated perfused adrenal glands; a very artificial situation. I therefore considered this a possibility and measured catecholamine levels before and after blockade by the nicotinic and muscarinic antagonists, hexamethonium bromide and (-) hyoscine methyl bromide, in dogs under pentobarbitone and chloralose-urethane anaesthesia.

Other evidence suggests selective release of noradrenaline occurs with increasing frequency of splanchnic nerve stimulation, (Klepping (1956), Malmejac (1957), Rapela (1956), Rapela and Covian (1954)). However, an equal amount of evidence has been produced suggesting either no change or even a decrease in the ratio of noradrenaline: adrenaline (Lund (1951), Malmejac (1964), Mirkin (1961)). As changes in the ratio are small and variable, evidence in favour of selective release is poor. However, the different techniques used by workers may be responsible for the differing results and different patterns of nerve stimulation may produce selective release of one catecholamine or the other. This theory in conjunction with the muscarinic and nicotinic work is discussed further in association with my own findings (see Discussion of Effects of cholinergic antagonists on the reflex release of catecholamines from the canine adrenal gland').

On the other hand, no evidence has been produced to support selective release during baroreceptor or chemoreceptor reflex stimulation. (De Schaepdryver (1959), Malmejac (1964), Critchley (1976)). This is not the case in the cat. Anichkov et al. (1960) compared the effects of carotid occlusion with cyanide injection, and found carotid occlusion produced a relatively greater vasoconstriction in a denervated hind limb whereas cyanide injection produced relatively greater constriction of a denervated nictitating membrane. For this reason Critchley and Ungar chose to investigate the release of adrenaline and noradrenaline in response to the baroreceptor and chemoreceptor reflex in cats and dogs.

For direct comparison with Critchley and Ungar's results we used the same adrenomedullary stimuli. For this we used a system which enabled us to independently alter the carotid perfusion pressure or the gas tensions of the blood perfusing the isolated carotid bifurcations. Stimulation to the carotid baroreceptors was reduced for periods of one minute and the chemoreceptors stimulated for one and a half minutes to produce roughly similar releases of catecholamines (adrenaline and noradrenaline) from the adrenal medulla.

As I have mentioned, there is some evidence to suggest nicotinic and muscarinic agonists may selectively release adrenaline and noradrenaline respectively from the canine adrenal medulla. The evidence supporting selective release in the cat is much firmer, (Douglas and Poisner (1965), Rubin and Miele (1968), Critchley (1976)). In the case of the cat however, adrenaline release is favoured by muscarinic stimulation and noradrenaline release by nicotinic

stimulation. These results, combined with the studies of Anichkov et al. (1960) and Critchley and Ungar (1974, 1975), reveal that both baroreceptor reflex stimulation and nicotinic agonists release noradrenaline from the cat adrenal medulla, whereas chemoreceptor reflex stimulation and muscarinic agonists release adrenaline. We may then hypothesise that the baroreceptor reflex in the cat is mediated by nicotinic receptors whereas the chemoreceptor reflex is mediated by muscarinic receptors. Support for this hypothesis comes from Critchley and Ungar's work, in which hexamethonium was found to abolish catecholamine release in response to baroreceptor reflex stimulation, while the response to chemoreceptor reflex stimulation was unaltered. We wondered therefore if a similar, or even opposite, situation exists in the dog. A physiological role for the nicotinic and muscarinic receptors, known to be present, being mediation of the baroreceptor or chemoreceptor reflex. We therefore investigated the response of the canine adrenal medulla to baroreceptor and chemoreceptor reflex stimulation, before and after the nicotinic and muscarinic antagonists hexamethonium bromide and (-)hyoscine methyl bromide. This also allowed us to investigate the effects of muscarinic and nicotinic antagonists on the release of noradrenaline relative to adrenaline from the canine adrenal medulla '*in vivo*'.

During a routine experiment by Ungar and Critchley, in which the release of catecholamines in response to chemoreceptor stimulation in the dog was being investigated, the sodium dithionite infusion into the carotid perfusion loop was inadvertently left on for 10 minutes. This was later found to have produced a marked release of catecholamines from the adrenal medulla which outlasted the stimulus by more than 30 minutes. This would suggest that prolonged carotid

body hypoxia evokes a humoral response which induces catecholamine release. The most likely source of a hormone acting on the adrenal medulla is obviously the adrenal cortex which completely surrounds the medulla in the dog. The cortex itself is under hormonal control from the anterior pituitary by corticotrophin and, as carotid body hypoxia has been shown to release corticotrophin from the anterior pituitary (Anichkov et al (1960), Marotta (1972)), it is reasonable to suppose that corticosteroids released from the cortex, by corticotrophin, may mediate the hormonally-induced catecholamine release.

It is known that high concentrations of glucocorticoids are necessary to maintain the synthesis of the enzyme PNMT, (phenylethanolamine-N-methyl transferase), which converts noradrenaline to adrenaline in the adrenal medulla (Pohorecky and Wurtman (1971)). It is this action of the corticosteroids which has most interested researchers, and any possible effect of corticosteroids on catecholamine release, rather than synthesis, has not been considered. However the work of Wurtman et al. (1968) contains several pieces of information which support the involvement of the pituitary-adrenocortical axis in modulating catecholamine release and encouraged us to investigate this possibility. The work of Wurtman et al. (1968) is discussed later along with our own results.

To investigate the possible involvement of the pituitary-adrenocortical axis in the release of catecholamines we studied catecholamine release in response to prolonged carotid body hypoxia in control dogs, dogs with denervated left adrenal glands and dogs pretreated with cycloheximide, a drug known to inhibit corticosteroid release in response to corticotrophin (Garren et al. (1965)). If the

release of catecholamines is humorally mediated then denervation should not affect release. If the pituitary-adrenocortical axis is involved then cycloheximide should abolish release. In addition to this study we also looked at the effects of Synacthen, a synthetic analogue of corticotrophin, on adrenomedullary release of catecholamines.

This has summarised the areas we intended to investigate during this research project. However, a chance finding opened out an alternative area of work which we also had the opportunity to investigate. One of the worst problems we encountered in studying the reflex release of adrenaline and noradrenaline from the canine adrenal medulla was large and maintained falls in systemic blood pressure following laparotomy. This is not generally considered a problem but the animals we were compelled to use, for financial reasons, were invariably in very poor condition. The animals used were mongrels coming largely from collie stock and it is possible that the breed of dog may have been partly responsible for the problems we experienced. To overcome the fall in blood pressure occurring following laparotomy we administered indomethacin, a prostaglandin synthetase inhibitor, as Terragno et al. (1977) have shown laparotomy releases prostaglandins, and that indomethacin given after laparotomy restores the blood pressure. We found indomethacin prevented the fall in blood pressure but in addition it also reduced adrenal blood flow. We therefore investigated the effect of indomethacin on adrenal blood flow and catecholamine release. For supportive data we investigated the effects of prostaglandin E_2 in the isolated perfused canine adrenal gland.

Our findings from these four areas of research,

A) Selective release of adrenaline and noradrenaline from the canine adrenal medulla.

B) Effects of cholinergic antagonists on the reflex release of catecholamines from the canine adrenal.

C) The role of the pituitary-adrenocortical axis in reflex responses of the adrenal medulla of the dog.

D) Effects of indomethacin on blood pressure, catecholamine release and adrenal blood flow in the anaesthetised, laparotomised dog.

are presented and discussed later.

The term 'catecholamine' in this thesis refers only to adrenaline and noradrenaline.

Parts of this work are published and copies of publications are included in the back of this thesis.

In the introduction I have outlined the work to be covered by this thesis and have described some of the findings which prompted the work. The physiology and pharmacology of the adrenal gland, relevant to this thesis, is discussed in relation to my own findings in the discussion sections. However, for a comprehensive review of all the physiology, pharmacology and biochemistry of the adrenal gland I refer the reader to 'Handbook of Physiology (1975) Section 7 Endocrinology VI Adrenal Gland' and to the very recent review article 'The regulation of the adrenal medulla' by Ungar and Phillips (1983).

I have however presented very brief summaries of the carotid baroreceptors and chemoreceptors concerning their relevance to the experimental methods I have used. I have also outlined the anatomy, innervation and blood supply of the adrenal gland before going on to consider the techniques available for the assay of catecholamines.

Arterial Baroreceptors

The baroreceptors are located in the carotid sinus, aortic arch, thyrocarotid junction and the cardiopulmonary area. They are stretch receptors which are excited by the expansion of the vessel wall, which is in turn controlled by blood pressure. With a normal blood pressure the baroreceptors exert a tonic inhibitory influence on sympathetic drive to the heart, blood vessels and the adrenal medulla. As blood pressure falls so does this inhibitory influence, accompanied by a corresponding increase in sympathetic activity resulting, among other things, in increased adrenomedullary secretion of catecholamines (Heymans and Neil (1958)).

The most accessible arterial baroreceptors are those located in the carotid sinus. For this reason we isolated an area around the carotid sinus and controlled the pressure within it. Apart from being the most accessible of the baroreceptor sites, the carotid sinus and the carotid body, which is densely populated with arterial chemoreceptors, may be isolated together in the same artificial perfusion loop.

To stimulate catecholamine release from the adrenal medulla we lower the perfusion pressure of the carotid sinus. To avoid referring separately to inhibition of baroreceptors and stimulation of chemoreceptors we have described the lowering of perfusion pressure as a 'baroreceptor test' and the response as the 'baroreceptor reflex'.

For a review of baroreceptors see 'Receptors under pressure' by A.M. Brown (1980).

Arterial Chemoreceptors

The systemic arterial chemoreceptors are located in the carotid bodies and in the aortic bodies. They are receptors which are sensitive to changes in the partial oxygen pressure (PO_2) of arterial blood and also to blood flow, but only when the PO_2 is low. In addition to the arterial chemoreceptors there are central chemoreceptors which are situated in the floor of the fourth ventricle and the medulla oblongata.

By isolating the carotid bifurcations we are dealing solely with arterial chemoreceptors. Therefore I use the term chemoreceptor in this thesis to refer specifically to the chemoreceptors of the carotid body. The lowering of the PO_2 of the carotid perfusate is referred to as a 'chemoreceptor test' and the response as the 'chemoreceptor reflex'.

For a review of arterial chemoreceptors see 'Arterial Chemoreceptors' by R.W. Torrance (Ed.) (1968) and 'The systemic arterial chemoreceptors' by M.J. Purves (Ed.) (1975).

The anatomy of the adrenal gland

The adrenal or suprarenal glands of mammals lie either separate from or attached to (primates) the superior surface of the kidney, as the terms adrenal and suprarenal imply. As it is the dog which has been studied for this thesis the anatomical details to follow will apply particularly to the dog and the term adrenal applied.

The adrenal glands are small, buff coloured, irregularly shaped endocrine organs. (3 cm x 1 cm x 0.5 cm in the dog). The right adrenal gland lies between the venacava and liver ventrally and the abdominal wall dorsally; its lateral border lying opposite the superior half of the right kidney. The left adrenal gland lies between the aorta and the medial border of the superior half of the left kidney, touching the abdominal wall dorsally. As seen with the kidneys the left adrenal gland lies inferior to the right and is considerably more accessible for dissection and cannulation procedures. Therefore, the left adrenal gland has been favoured by us and others for investigation *in vivo*.

The adrenal gland is composed of an outer cortex and an inner medulla bound by a thin capsule, consisting largely of connective tissue. The cortex, developed from cells of the coelomic mesothelium, is sub-divided into three clearly distinguishable zones, the zonae glomerulosa, fasiculata and reticularis. Although some conflicting evidence exists, the zona glomerulosa probably produces the mineralocorticoids, desoxycorticosterone and aldosterone, which maintain the electrolyte level in extracellular fluids. The function of this zone is dependent only on sodium and potassium balance and is

independent of the hypothalamus and pituitary. The zona fasiculata and zona reticularis produce glucocorticoids, the main ones being corticosterone, hydrocortisone (cortisol) and cortisone. (The zona fasiculata is thought to be non-secretory, acting as a store of steroid precursors for the zona reticularis). The glucocorticoids have many functions, among them are facilitation of protein catabolism and gluconeogenesis, destruction of lymphocytes and release of gamma globulins (increased immune response) and inhibition of cellular and fibrous proliferation (anti-inflammatory). The function of these two zones is closely controlled by the hypothalamus (corticotrophin releasing factor, CRF) and the pituitary, (adrenocorticotrophic hormone, ACTH).

The adrenal medulla, in contrast to the cortex, is developed from ectodermal cells derived from the neural crest. The medulla is red/brown in colour, making it clearly distinguishable from the pale yellow cortex surrounding it. The medulla is composed of a spongework of large polygonal cells, separated by sinusoidal vessels. The cells of the medulla are granular, the granules being evenly distributed in the cytoplasm and containing the catecholamines of the adrenal gland (Hillarp and Nilsson, (1953, 1954) ; Blaschko and Welch (1953)). The medullary cells stain brown with fixatives containing chromium salts, due to an oxidation and polymerisation of the catecholamines. The cells are therefore referred to as chromaffin cells (Kohn 1898) or phaeocromocytes (Poll 1906).

The adrenal medulla was first shown to contain noradrenaline as well as adrenaline by Holtz et al. (1947). Since then, noradrenaline has been identified in the adrenal glands of many different species in

varying proportions. After the discovery of noradrenaline in the adrenal medulla, Bander (1950, 1954), using classical staining techniques, was able to show two types of medullary cell present in the adrenal glands of dogs, cats and mice. Bander (1950, 1954) proposed that the two cell types were related to the production of adrenaline and noradrenaline. Support for this proposal came from Eranko (1952) who demonstrated acid phosphatase positive (adrenaline) and acid phosphatase negative (noradrenaline) cell types in the adrenal medulla. Eranko (1954, 1955, 1958) and Hillarp and Hokfelt (1954) then went on to confirm that there were indeed separate adrenaline and noradrenaline containing cells in the medulla. Bander's (1950, 1954) original proposal was validated but it was later shown by Eranko and Palkama (1959) that the differential staining seen by Bander (1950, 1954) was dependent only on the concentration of catecholamines present and not on the type.

In ruminants, as distinct from other domestic animals, two separate zones are found in the adrenal medulla. An outer zone which stains dark brown with chromium salts and produces adrenaline and an inner zone which stains yellowish and produces noradrenaline. This zonation occurs as a result of conversion of noradrenaline to adrenaline requiring the enzyme phenylethanolamine-N-methyl-transferase (PNMT). PNMT is dependent on the high concentration of adrenocorticosteroids for its activity and the dark outer zone reflects the area supplied with blood from the adrenal cortex.

Innervation of the adrenal gland

The adrenal medulla can properly be regarded as a specialised ganglion of the sympathetic nervous system and the secretory chromaffin cells are richly innervated by cholinergic preganglionic sympathetic nerve fibres derived from the lower thoracic segments of the spinal cord. The main innervation comes from the greater splanchnic nerve which usually leaves the sympathetic trunk at the twelfth thoracic sympathetic ganglion. It varies in its origin, however, from the eleventh thoracic to the first lumbar ganglion. The largest of the splanchnic nerves, it divides into many branches on approaching the dorsum of the adrenal gland. Some of these end in small adrenal ganglia, others enter the gland directly, while the identity of others is lost in the coeliac plexus. A minor contribution to the innervation of the adrenal medulla comes from the lesser splanchnic nerves of which there are generally two, usually leaving the thirteenth thoracic and first lumbar sympathetic ganglia. Frequently these ganglia are fused, conversely they may be widely separated. The lesser splanchnic nerves run to the dorsum of the adrenal gland where they branch and become part of the adrenal plexus.

Teitelbaum (1934) suggested the possibility of a vagal contribution to the innervation of the adrenal gland but electrophysiological experiments have not been able to support this. Some preganglionic vagal fibres are dispersed in the adrenal plexus but do not synapse there and in the main go on to innervate the small intestine via the superior mesenteric ganglion.

No nerve supply to the adrenal cortex has been demonstrated.

Blood Supply to the Adrenal Gland

The adrenal gland derives its blood supply from many very small adrenal arteries which originate from the nearby large arteries, usually the renal artery, inferior phrenic artery and aorta. The vessels form a capsular plexus in the adrenal gland from which capillary vessels pass centripetally between and around the columns of cortical cells. A few arterioles pass deep into the cortex (arteriae corticis) or the medulla (arteriae medullae) before breaking up to form capillaries. Thus, some blood can reach the adrenal medulla without first passing through the capillaries of the cortex. Blood then flows between the chromaffin cells of the medulla and is collected in the venae medullae which fuse to form the adrenal vein. On leaving the gland the adrenal vein fuses almost immediately with either the renal vein or the inferior vena cava.

The existence of two pathways to the adrenal medulla has led to speculation as to whether the blood supply via the cortical capillaries can be adjusted to regulate the concentration of glucorticoids bathing the adrenal medullary cells. In support of this Symington (1962) has described smooth muscle bundles flanking the openings of the cortico-medullary venous sinusoids.

For a review of the blood supply to the adrenal gland see "The Natural History of the Chromaffin Cell" by R.E. Coupland (1965).

Techniques available for catecholamine assay

Four basic methods are currently employed for the assay of catecholamines. They are:-

1. Biological assay

   (see Gaddum (1959), Vane (1966) and Callingham (1967)).

2. Chemical techniques

   These techniques are dependent on the formation of fluorescent derivatives from catecholamines.

   (see Udenfriend (1962), Häggendal (1966) and Callingham (1967)).

3. Electrochemical detection

   This is dependent on the 2 electron oxidation of catecholamines to form o-quinones. This generates a current, proportional to the concentration, which is measured.

   (see Kissinger (1977) and Ryan and Wilson (1979)).

4. Isotope derivative techniques

   These depend on enzyme specificity and the formation of radio-labelled derivatives.

(see Engelman and Portnoy (1968, 1970) and Passon and Peuler (1973))

Of these techniques bioassay has changed the least during the last 20 years. In the review of Gaddum (1959), he suggested that, in comparison to chemical methods, "there is little doubt that as time goes on biological methods of assay will be less used, but they are still important and chemical methods will only inspire universal confidence if they are shown to give the same results as the biological methods." This prediction has proven to be true with chemical methods now largely used. However, chemical, fluorimetric, methods have reached the stage bioassay was at 20 years ago, where little development of the technique is now taking place. Chemical techniques have reached their limit and more sensitive methods are being sought for circulating and brain catecholamines, e.g. isotope derivative techniques and electrochemical detection.

My requirements of the catecholamine assay are as follows:-

1. Reasonable sensitivity and specificity; as adrenaline and noradrenaline are the major catecholamines in adrenal venous blood and are present in fairly large quantities.
2. Reliability and reproducibility.
3. Good discrimination between adrenaline and noradrenaline.
4. High throughput.
5. Low cost.
6. Simple assay, requiring only one operative.

I shall briefly discuss each of the four techniques with these requirements in mind.

Bioassay

The sensitivity of most bioassay techniques is good, e.g. less than 1ng of adrenaline or noradrenaline (Booker, (1959)). However, the specificity is often very poor and many substances will interfere. These substances may have a catecholamine like action on the tissue themselves or may potentiate or attenuate the effects of catecholamines, e.g. 5-hydroxytryptamine potentiates the vasoconstriction caused by catecholamines in the rabbit ear artery. (De la Lande and Harvey, (1965)).

The best known preparation for discriminating between adrenaline and noradrenaline is the isolated rat uterus pretreated with stilboestrol. (Gaddum, Peart and Vogt, (1949)). This preparation is about 100,000 times less sensitive to noradrenaline than adrenaline. An honours course practical conducted by the University of Edinburgh is the differential assay of adrenaline and noradrenaline using the superfused rat stomach strip in series with the rat uterus. By comparing the reactions of the two tissues to the unknown mixture with the reactions to adrenaline and noradrenaline, the proportions of each in the mixture can be calculated by solving a pair of simultaneous equations. On testing standard mixtures of adrenaline and noradrenaline the technique was only able to 'roughly' determine the concentrations of adrenaline and noradrenaline. This was concluded from several assays in which reliability and reproducibility also proved poor (possibly due to unfamiliarity of the students with the technique).

Bioassay is certainly cheap and easy and does have a high throughput. However, in view of the other considerations bioassay has not been used.

The best use of bioassay for the assay of adrenal venous blood would be 'on-line', i.e. Cascade superfusion of tissues directly with adrenal venous blood. Catecholamine secretion would therefore be determined independent of any changes in adrenal blood flow. In addition, owing to the high concentrations of catecholamines, the blood could be diluted as it leaves the gland and the interference produced by other substances disregarded. An 'on-line' superfusion was not attempted owing to limited man-power. However, if several demonstrators were available, this could make a very interesting experiment for teaching purposes.

Chemical techniques

The fluorimetric estimation of catecholamines fulfils all of my requirements and is the method of choice. However, many variations of this method exist. The selection and modification of techniques used is discussed later.

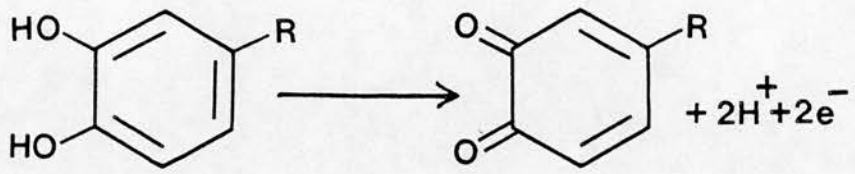
Electrochemical detection

Electrochemical detection used in conjunction with high pressure liquid chromatography (HPLC) has been gaining popularity since its first practical application in 1973; the assay of catecholamines in brain tissue, (Kissinger et al, (1973)). Following this initial publication, liquid chromatography with electrochemical detection (LCEC) has been applied to a wide variety of problems, involving the trace detection of

easily oxidised (or reduced) substances; among them catecholamines in urine (e.g. Kissinger et al, (1975)) and plasma, (e.g. Hallman et al, (1978)).

The fundamental technique in electrochemical detection is voltammetry. In this process, an electrode is polarised (held at a fixed potential with respect to a reference electrode) at a potential at which a desired chemical reaction occurs; in this case the two electron oxidation of adrenaline and noradrenaline to o-quinones.

e.g.



The resulting current which flows in the circuit is dependent upon the rate of the chemical reaction taking place at the electrode surface. Since the rate is governed by the concentration of the substance taking part in the reaction, the measured current is proportional to concentration, i.e. the current is a function of concentration at a fixed potential. When using electrochemical detection, in which the solution flows continuously through the detector cell rather than remaining stationary, as in voltammetry, it is essential the flow rate remains absolutely constant. This ensures the resulting peak in the current signal is directly proportional to concentration and not due to changes in flow.

The equipment required for LCEC is expensive. Even being fortunate working in a department which possesses a good constant flow HPLC machine, the commercially available electrochemical detector and potentiostat-amplifier will still cost between £450 and £2,000 (May 1979 price lists - Anachem Ltd. and EDT Research). This would place this technique beyond our means. However, many investigators are using their own 'home-made' detectors and amplifiers, for which methods of construction are published. (Cost is about £20). In view of this and the reported high sensitivity, simplicity and low running costs of the method I decided to further explore the LCEC technique - I visited Dr. Sharman of the A.R.C. Research Institute, Babraham who routinely uses LCEC for the assay of catecholamines and their metabolites in brain tissue. Whilst not convinced the technique was any better than fluorimetry for the assay of adrenal venous catecholamines, he did not discourage me from attempting it, and provided me with several useful tips for setting up the assay in Edinburgh.

The apparatus was assembled and tested using standard mixtures of adrenaline and noradrenaline. (See later for details). The sensitivity of the method was good, (100pg) but in all other requirements the technique fell short of fluorimetry. Hence, fluorimetry rather than LCEC was used for the assay of adrenal venous catecholamines.

A full account of my investigation into LCEC is presented later.

Isotope derivative techniques

This technique is currently the most favoured for the assay of catecholamines in peripheral plasma, especially by clinical departments

assaying catecholamines in human plasma.

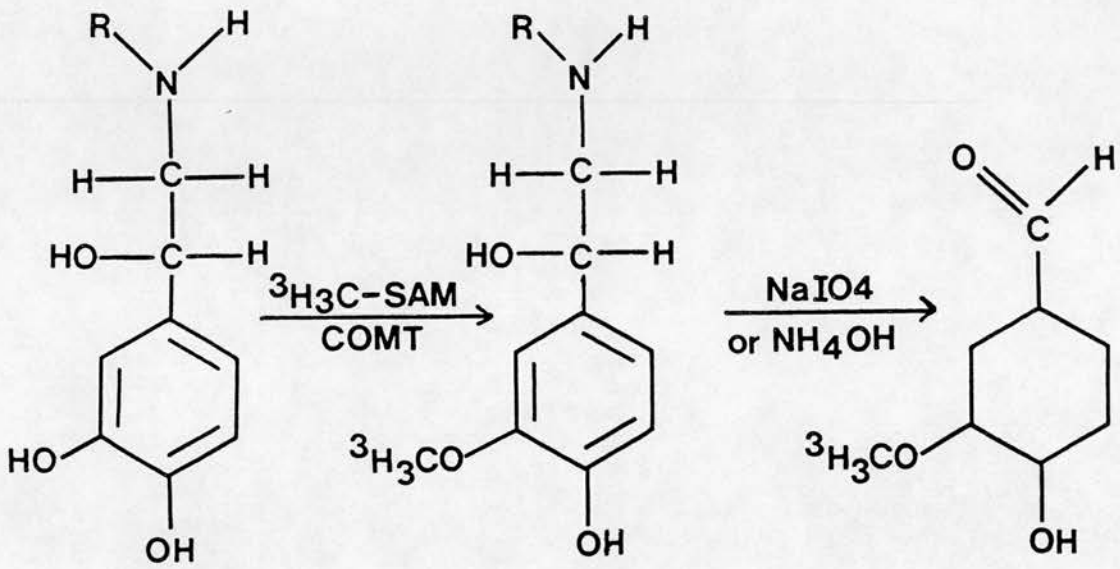
The original double isotope derivative method of Engelman et al (1968, 1970) was considered a major methodological advance in the field of catecholamine analysis. The method is based on the enzymatic conversion of catecholamines with S-adenosyl-L-methionine. (methyl[^{14}C])(H_3 ^{14}C-SAM) and catechol-O-methyltransferase to their 3-O-methylated derivatives. However, the precision of this assay was unsatisfactory and the adrenaline concentrations in human plasma were at the detection limit of the assay. Passon and Peuler (1973) introduced an important modification of this method, the single isotope derivative method, by using $^{3}H_3$C-SAM which has a considerably higher specific activity than H_3 ^{14}C-SAM. This resulted in a 10-fold improvement of the assay sensitivity so that the sample volume could be reduced from 10ml to less than 1ml plasma. The losses were corrected for by use of internal standards.

The single isotope derivative method for measuring catecholamines in plasma is now the technique generally used but does exist in many modified forms, e.g. da Prada and Zürcher (1976), Peuler and Johnson (1977), Nagel and Schümann (1980), Bosak et al (1980).

The two major steps in the radioenzymatic catecholamine assay are

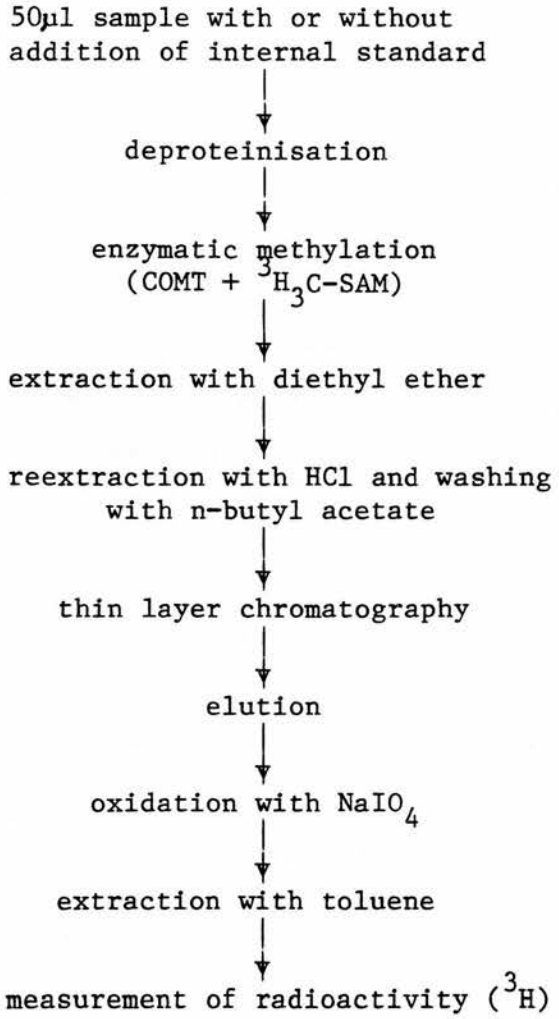
1. conversion of adrenaline and noradrenaline to metanephrine and normetanephrine respectively, by catechol-O-methyltransferase (COMT) using $^{3}H_3$C-SAM as the methyl donor and,

2. subsequent oxidation of the 3-O-methyl derivatives to vanillin, by either sodium periodate or ammonium hydroxide, e.g.



R = CH_3	ADRENALINE	METANEPHRINE	VANILLIN
R = H	NORADRENALINE	NORMETANEPHRINE	

As the reagents for this technique are very expensive and all samples are done in duplicate, one sample used for the addition of internal standards, the cost exceeds £2 per sample. This precludes us from even considering this technique for our purpose. Therefore, rather than discussing all the possible modifications I have presented a typical working protocol (taken from Bosak et al, 1980) which outlines the procedures involved and demonstrates the slow, tedious nature of this technique:-



In a recent publication, (Uchikura et al (1981)), an attractive modification of the method has been described, using ion-pair liquid chromatography to separate the radio-labelled 3 and 4-O-methylated derivatives. This method avoids the tedious and cumbersome manipulations of periodate oxidation, solvent extraction and thin-layer chromatography whilst still providing an accurate and sensitive method for the determination of catecholamines in blood. This method was not published when I established my assay but the high cost would still have prevented my adopting this technique.

Methods

Investigation of Electrochemical Detection with High Pressure Liquid Chromatography for the assay of catecholamines

A brief account of the principles of electrochemical detection is already presented. (see 'Techniques available for catecholamine assay'). For a fuller account Kissinger, 1977 and Ryan and Wilson 1979 are recommended.

Equipment

1. High pressure liquid chromatography (HPLC)

Two types of chromatography were evaluated,

a) Cation exchange chromatography, for which a commercial 'Partisil SCX' (strong cation exchange) column was used. The eluant is an acetate-citrate buffer (pH 5.2) which is prepared from 5.75g citric acid (monohydrate), 6.8g sodium acetate.$3H_2O$, 1.05ml glacial acetic acid and 2.4g sodium hydroxide diluted to 1 litre. (see Keller et al 1976). The eluant is pumped at a constant rate of 0.5ml/min.

b) Reverse-phase (soap) chromatography, for which a metal column packed with ODS/TMS silica was used. The eluant in this case comprises 50mg sodium lauryl sulphate (SLS) dissolved in a solution of 600ml. 0.1M citric acid and 320ml of 0.1M disodium

monohydrogen orthophosphate. The eluant is taken from Kissinger and Riggin (1977) with SLS used as the detergent. The eluant is pumped at a constant rate of 0.5ml/min.

The detergent used in 'soap' chromatography is absorbed by the reverse-phase surface to form a layer which in some ways is akin to an ion-exchanger.

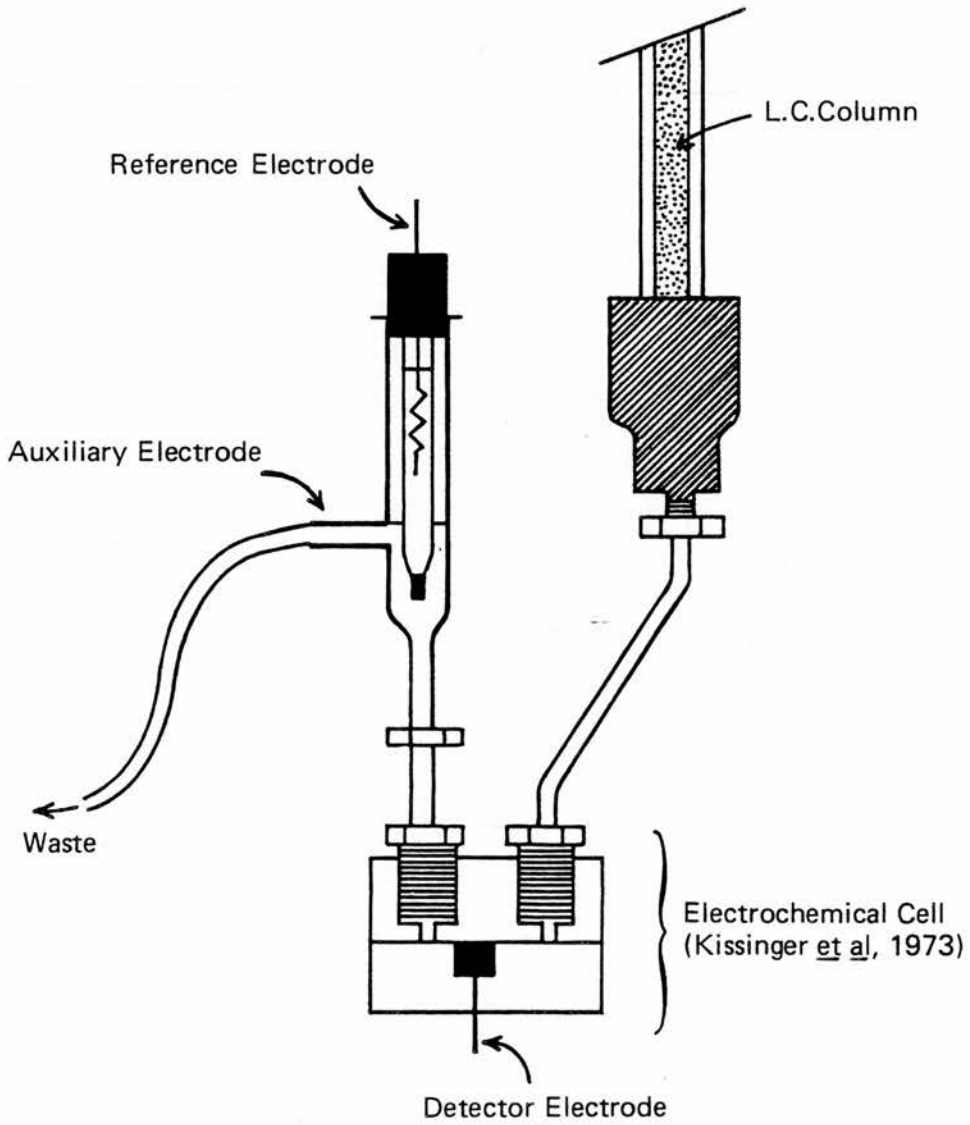
For this reason the column is primed with eluant for some time before attempting to run samples. SLS is chosen as the detergent as it can form an ion-pair with the ionised form of the solute (catecholamines). To ensure that the catecholamines are in the preferred ionic form the pH of the solution is made acidic. Thus, for catecholamines, an acidic eluant containing a cationic detergent, as above, is generally used.

A good review of the separation of catecholamines by HPLC is given by Knox and Jurand, (1976), of the Wolfson Liquid Chromatography Unit, University of Edinburgh.

2. Detector electrode

The detector electrode holder, or block, made according to the method of Kissinger et al, (1973), is connected to the outflow of the HPLC column (see figure 1). The electrode itself is made of graphite paste which is prepared by thoroughly mixing 3.25g of graphite (high purity - supplied by Dr. Sharman) with 1.75g of Dow

Figure 1 A schematic representation of the thin-layer electrochemical detector used with high pressure liquid chromatography



Corning high vacuum silicone grease. The graphite paste is then tightly packed into the cavity around the copper wire of the detector block and left slightly proud. The final smooth surface is formed by rubbing the block on the surface of a computer index card laid on glass (this ensures an even surface). The smoother the surface of the electrode the less the electrical noise.

3. Reference and Auxiliary electrodes

In addition to a detector electrode one other electrode is required so that an electrical circuit can be completed. In practice it is usual to use two additional electrodes; a reference and an auxiliary electrode. The auxiliary electrode is included to ensure that no current flows through the reference electrode, ensuring a constant potential is applied between the detector electrode and the reference electrode. Without the auxiliary electrode the reference electrode must conduct current flowing in the cell, therefore the potential of the reference electrode cannot be truly constant. Also, if the resistance of the solution in the cell is high, a potential drop will occur at the electrode/solution interface. The reference electrode used was a commercial 'saturated calomel electrode' and the auxiliary electrode simply an inert metal outflow tube from the reference electrode cell. The reference electrode cell and holder are constructed from a 10ml plastic syringe with the auxiliary electrode fused in one side. (see figure 1)

4. Potentiostat-Amplifier

A schematic of the potentiostat and current amplifier is shown in figure 2. This was constructed by Mr. P. Whelpdale of the Department of Pharmacology, University of Edinburgh and is a modification of the system used by Keller *et al* (1976).

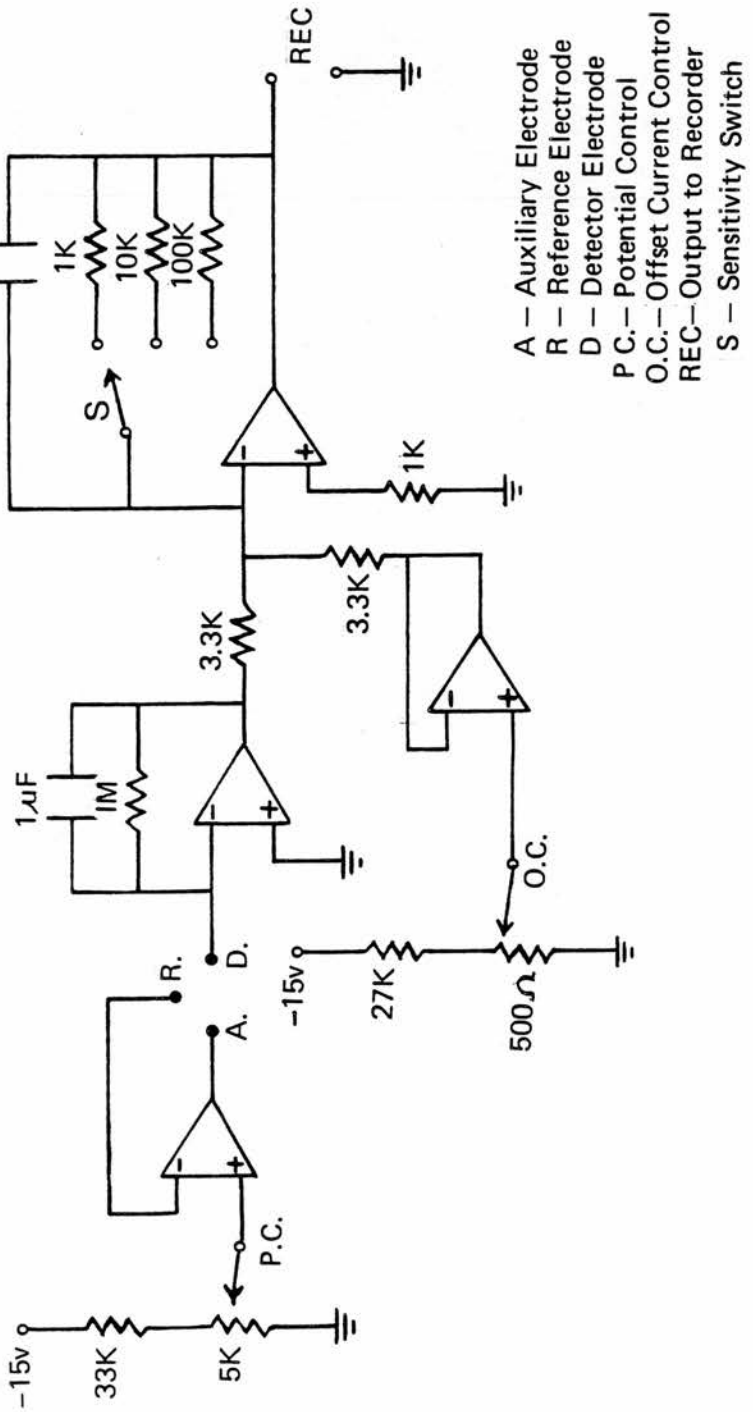
The potential of -15V is applied for all catecholamine work but can be reversed to +15V if reduction reactions are involved. The potential control is adjusted until the detector lead is +0.5V with respect to the reference lead. A higher potential than this will increase background current whereas a lower potential will not be sufficient to oxidise the catecholamines and will result in reduced sensitivity.

When connecting the electrodes it is imperative that the reference and auxiliary leads be connected before the detector lead. Failure to do this will result in destruction of the electrode surface giving a very noisy signal, necessitating repacking of the electrode.

5. Ultra-violet absorption spectrometer

To identify the time course of catecholamine elution and column characteristics an ultra-violet absorption spectrometer with a built in flow cell was coupled onto the column in place of the

Figure 2 Potentiostat – Amplifier Circuit



electrochemical detector. This also provided an alternative detector with which to compare the system under test.

Setting-up procedure

1. The eluant is vacuum deaerated before use and is kept on a hotplate ($45^{o}C$) to ensure minimum bubble development in the detector.

2. A coil of 1/16" (o.d.) teflon tubing is connected between the constant flow pump and the column to dampen any oscillations in flow. If this is omitted the baseline will resemble a sine wave pulsing in time with the pump. - This clearly demonstrates the dependence of the detector on a constant flow.

3. The column is primed with eluant at 1ml/min. for 2 hours to ensure stable conditions within the column (particularly when 'soap' chromatography is being used).

4. The electrochemical cell is filled with eluant from a syringe before attaching it to the column. This prevents bubbles from becoming trapped in the detector. If bubbles are present then serious 'noise' problems will occur; the bubbles must be removed before continuing.

5. Flow is reduced to 0.5ml/min.

6. The reference cell is connected and the outflow run to waste.

7. The potentiostat-amplifier is connected to the 3 electrodes; the detector electrode being connected last.

8. The electrode is left for up to 5 hours to stabilize to low residual currents. When used electrodes are replaced in the system the stabilization time is much less, however, with reused electrodes the noise level increases significantly. Ideally the electrode should be set up, stabilized and not removed from the set-up until replacement is required (normally after about 3 months of continuous usage).

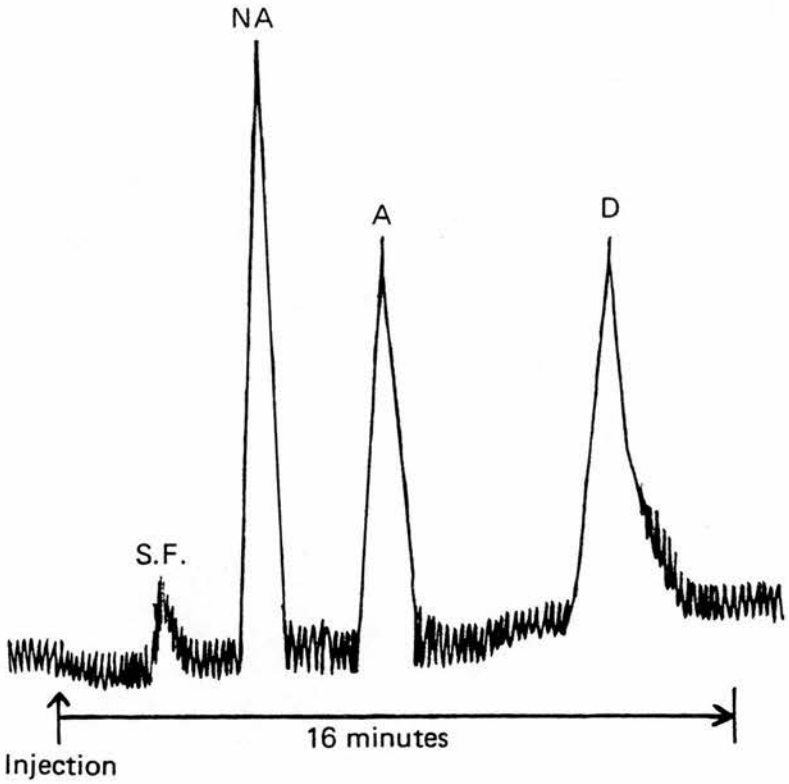
9. All standards of adrenaline and noradrenaline are made up and injected in 10^{-4}M HCl.

Results

1. Type of chromatography:-

Both cation exchange and soap chromatography separated the catecholamines adrenaline and noradrenaline completely. However, under the conditions described the separation was better using soap chromatography. In addition, dopamine is separated from adrenaline using soap chromatography but not by cation exchange chromatography.

Figure 3 Tracing of a chromatograph obtained using SOAP Chromatography with electrochemical detection.



Chromatograph of a 10µl injection containing 500 pg of noradrenaline (NA), adrenaline (A) and dopamine (D).

(Solvent Front = S.F.)

Figure 3 is an example of a chromatograph obtained using soap chromatography with electrochemical detection.

2. Limit of sensitivity:-

Three different detectors were evaluated for their limit of sensitivity, i.e. The lowest concentration of adrenaline and noradrenaline in a standard mixture containing equal concentrations of each which consistently gives peak heights significantly greater than the background noise.

a) Ultra-violet absorption:- As expected this produced a poor limit of sensitivity, only 20ng.

b) Electrochemical detection (Home-made using a carbon paste electrode):- 100pg could be detected by the better made electrodes but most could only cope with 200-300pg.

c) Electrochemical detection (EDT Research glassy-carbon electrode):- Replacing the carbon paste electrode with a commercial glassy-carbon electrode caused the sensitivity to fall with the limit being about 2ng. EDT Research claim limits well below 500pg for clean catecholamine standards; however, a representative of the company was unable to substantiate these claims using his equipment in our laboratory. In fact, using the EDT potentiostatic control

unit (£1,500) rather than our own (£15) decreased sensitivity further; the limit rising to 5ng.

3. Linearity:-

The assay was tested over the range 100pg to 10μg adrenaline and noradrenaline and found to be linear.

4. Specificity:-

Electrochemical detection is not itself a highly specific assay as several compounds will oxidise at the applied potential. However, using HPLC in conjunction with electrochemical detection results in a very selective assay.

Discussion of problems arising when using this assay for the determination of adrenaline and noradrenaline in adrenal venous blood

This assay technique is used for the assay of catecholamines in blood (Hallman et al 1978) and therefore any problems which may arise with the assay per se can be overcome. However, what interested me was the problems this assay would create for me personally compared to any alternative techniques, especially fluorimetry.

The assay itself would fulfil all my original requirements with the sensitivity better and the throughput perhaps a little lower than that desired. I have not designed any purification and extraction procedures to preceed the assay but I do envisage one potential problem:- The department does not allow strong acids to be used with the HPLC machine, i.e. the catecholamines would have to be prepared finally in weak acid. This would result in a dilution being necessary and sensitivity lost. Boric acid is not suitable for chromatography so my extraction technique for fluorimetry would not be of any use here. Other extraction procedures use hydrochloric acid to elute catecholamines from strong cation exchange resins, e.g. 4mls of 1N HCl elutes from Amberlite CG-120 (see 'Notes on catecholamine assay'). If this eluant was then diluted to 0.1N the sample would be contained in 40mls. The maximum volume I can inject is 100µl i.e. 1/400th of the sample is assayed and with a sensitivity limit of 500pg the sample limit would be 200ng. This is well below the sensitivity of most other techniques.

In addition to this the HPLC machine is required by other users and at most I could hope for three days of uninterrupted use. This would necessitate using a new electrode each time, resulting in varying sensitivities between batches and many hours spent in preparation and setting-up.

As I could not obtain an HPLC for my exclusive use, and the fluorimetric assay (which was developed in parallel with this) appeared to fulfil all requirements perfectly, this technique was not investigated further. However, if used routinely, in experienced hands this assay could provide a much simpler alternative than the isotope derivative technique for the assay of catecholamines in peripheral plasma.

NB:- When investigating this technique I did not enclose the equipment in a Faraday cage. This resulted in high background noise and the limit of sensitivity could undoubtedly be lowered by the inclusion of a Faraday cage in the apparatus.

Chemical Spectrophotofluorimetric Catecholamine Assay

The objective was to establish a simple and reliable physicochemical technique for the assay of adrenaline and noradrenaline in adrenal venous blood.

Adrenaline and noradrenaline are present in adrenal venous blood in high concentrations with relatively little contamination. Thus, a simple extraction procedure is sought which will yield high and consistent recoveries. The sensitivity of the assay is not a major concern as the concentration of catecholamine is high in adrenal venous blood. However, in some experiments, especially denervated adrenal gland experiments, the catecholamine levels are reduced and therefore, whilst not a major concern, a good sensitivity is desirable.

It is important for the assay to differentiate adequately between adrenaline and noradrenaline as 'selective release' of catecholamines is investigated.

Finally, as large numbers of samples are taken during each experiment (up to 35), a high throughput assay is sought; preferably with large batch handling as comparison of samples within a batch is often more reliable than between batches.

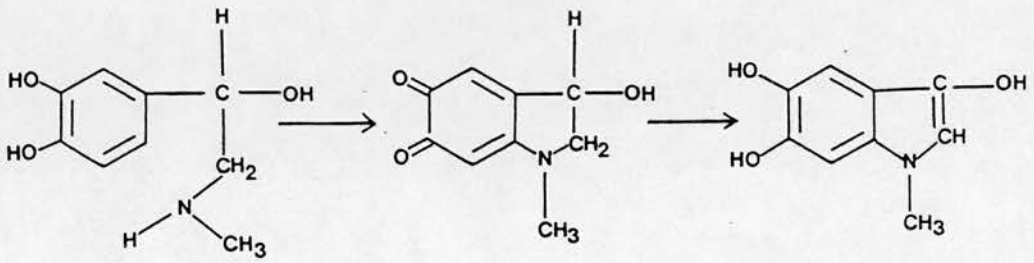
Selection of technique:-

Physico-chemical techniques for the assay of adrenaline and noradrenaline are based on the observation that adrenaline and noradrenaline, and particularly their oxidation products, fluoresce. Vulpian initially described the property of the adrenal gland to form lightly coloured compounds and Loew (1918) observed that adrenaline, in strong alkali, produces a yellow-green fluorescence.

Later, in 1930, Paget showed this fluorescence was specific for adrenaline. Gaddum and Schild (1934) showed the reaction required the presence of oxygen, and noradrenaline, which also produced fluorescence, gave a much weaker reaction than adrenaline. Gaddum and Schild (1934) attempted to use this reaction for assay purposes but were largely unsuccessful owing to the transient nature of the fluorescence. The breakthrough in the fluorimetric assay of adrenaline and noradrenaline came in 1948 when Ehrlen stabilised the adrenaline fluorophor by adding ascorbic acid. Based on this principle, Lund (1950) developed the method and adapted it to produce the first fluorimetric estimation of adrenaline and noradrenaline in blood.

The reaction which occurs involves the oxidation of adrenaline and noradrenaline to the cyclised adrenochrome and noradrenochrome respectively. (Richter (1937) and Richter and Blaschko (1937)). The coloured 'chrome' derivatives are then isomerically transformed by the addition of alkali to form 'lutines'; the term first used by Lund (1949). It was the work of Ehrlen (1948), Lund (1949), Fischer (1949)

and Harley-Mason (1948, 1950) which finally identified the fluorescent derivative of adrenaline as 1-methyl-3,5,6-trihydroxyindole (adrenolutine). Thus, the reaction became known as the 'trihydroxyindole' (THI) reaction.



Adrenaline Adrenochrome Adrenolutine

The differential assay for adrenaline and noradrenaline used by Lund (1950) was based upon the pH controlled differences in the oxidation rates of these amines. This requires splitting the sample and oxidising with manganese dioxide at pH 3.0 and 6.5 for the oxidation of adrenaline and noradrenaline respectively. - The sensitivity of this method was poor. A major improvement came in 1957 when Price and Price differentiated between the lutines of adrenaline and noradrenaline by using light at two wavelengths rather than oxidation at two different pH levels. Furthermore, Price and Price (1957) used potassium ferricyanide as the oxidising agent and reacidified the reaction products from the alkaline reaction to pH 5.0. This increased the fluorescence intensity and produced stable fluorophors. (see 'Notes on assay')

Since 1957 the number of variations of the THI technique has mushroomed and are reviewed in detail by Udenfriend (1962), Häggendal (1966) and Callingham (1967). The major difference between the various techniques lies in the choice of oxidising agent and stabilizing reagent used.

e.g.

Oxidising agents

a) Manganese dioxide e.g. Lund (1950), Cohen and Goldberg (1957), Pekkarinen (1954), Crawford and Law (1958).

b) Iodine e.g. Crout (1961), Laverty and Taylor (1967), Sourkes and Drujan (1957).

c) Potassium ferricyanide e.g. Price and Price (1957), Bertler et al (1958), Vendsalu (1960), Anton and Sayre (1962), Häggendal (1963), Valori et al (1970), Euler and Lishajko (1959).

Stabilizing reagents

a) Ascorbic acid e.g. Lund (1950), Price and Price (1957), Cohen and Goldberg (1957), Crout (1961), Anton and Sayre (1962).

b) Sulphur containing compounds:-

1. British anti-Lewisite (BAL)/Formaldehyde e.g. Valori et al (1970).
2. BAL/Sodium sulphite e.g. Häggendal (1963).
3. Sodium sulphite e.g. Laverty and Taylor (1967).
4. Cysteine hydrochloride e.g. Klensch (1966).
5. Thioglycolic acid e.g. Merrills (1963).

An alternative method to the THI reaction is the ethylenediamine condensation reaction. In 1949 Natelson, Lugovoy and Pincus found that catecholamines formed strongly fluorescent condensation products with ethylenediamine. Weil-Malherbe and Bone (1952) applied this method to plasma extracts, purified by alumina adsorption, and obtained stable fluorescent products. By measuring the fluorescence at two wavelengths, with the use of differential secondary filters, the concentration of adrenaline and noradrenaline was determined. Several modifications of this method have been tried; (see Weil-Malherbe, (1959) and Manger, Wakim and Bollman, (1959)) however, the ethylenediamine condensation reaction appears to be less specific than the THI reaction. (Holzbauer and Vogt, (1954), Euler and Floding, (1955), Euler, (1956), Valk and Price, (1956) and Cohen and Goldenberg, (1957)).

The reaction I have used is the THI reaction. The actual method employed has its origins in Bertler, Carlsson and Rosengren (1958) with modifications by Häggendel (1963) and Valori, Brunori, Renzini and Corea (1970). I have selected from these various techniques and modified them to suit my own purpose (for details see 'Notes on assay').

Selection of Extraction technique

Before the THI reaction is applied the sample must first be purified.

Three extraction techniques for catecholamines are commonly used, solvent extraction, adsorption onto alumina and cation exchange chromatography.

The first, solvent extraction, (introduced by Weil-Malherbe and Bone (1954)), is usually applied to biological tissues rather than fluids. However, solvent extraction without further purification is seldom adequate for the fluorimetric estimation of catecholamines. (e.g. Chang (1964)). Therefore, solvent extraction may be deemed redundant.

Of the other two techniques, cation exchange chromatography is by far the simplest to use and is the technique I have used here. I have used a weak carboxylic acid resin, Amberlite CG50, (Bergström and Hansson (1951), Kirshner and Goodall (1957)) rather than a strong acidic resin of the sulphonic acid type (e.g. Bertler et al, (1958), Häggendal (1962, 1963)) - see 'Notes on assay" for details.

The extraction procedure depends on the positively charged catecholamines (at neutral pH) being retained by the negatively charged carboxylic groups on the resin. The catecholamines are eluted from the resin using boric acid.

EXPERIMENTAL PROTOCOL FOR CATECHOLAMINE ASSAY

a) Adrenal venous samples

Collection of sample

Heparinised blood from the left adrenal gland is collected in cooled, graduated centrifuge tubes. The tubes are stored in ice until 2, 3 or 4 are collected.

Separation and storage of plasma

The tubes are immediately centrifuged at 3,000r.p.m., 4°C for 5 minutes.

The plasma supernatant and packed red cell volumes are recorded and the plasma carefully removed using a pasteur pipette.

1mg/ml of sodium metabisulphite is added to the plasma and the sample frozen at -20°C for up to 2 weeks.

Preparation of plasma for extraction

The plasma is thawed and centrifuged, 3,000r.p.m., room temperature for 2 minutes to remove precipitated fibrin.

* From here duplicates of each sample are taken *

To 1ml aliquots plasma is added 3mls of 0.1N phosphate buffer, pH 7.4 and 0.5mls of 3% EDTA. (If 2mls of plasma are not available the whole sample is used and diluted as above, in the ratio 1:3:05. The sample is then split to provide duplicates).

Extraction and purification of catecholamines

The samples are loaded onto Amberlite CG50 (mesh size 100-200) resin columns of 5cm length and 0.25cm diameter.

The column is rinsed using 5mls of distilled water followed by 0.15mls of 2/3M boric acid.

The catecholamines are then eluted with 1ml of 2/3M boric acid and collected in test tubes designed to fit both the AC60 autoanalyser and the spectrophotofluorimeter.

The eluted sample has a pH of 6.5 and is stable at room temperature for longer than 3 hours.

b) Column blanks

A peripheral blood sample is taken via the femoral artery and substituted for adrenal venous blood in the above protocol.

6 column blanks are processed.

c) Column standards

Standard solutions of adrenaline and noradrenaline as 1µg/ml in 10^{-3}M HCl are prepared.

0.1ml of each standard is added, in duplicate, to 4 of the column blanks at the dilute stage, immediately prior to loading onto the column. The column standards then follow the rest of the protocol.

Following elution the tubes are placed in the moving conveyor belt of a Unicam AC60 autoanalyser in strict order.

Ahead of the samples are placed duplicate non-column blanks and standards.

Non-column blanks:- 1ml of 0.1N phosphate buffer, pH6.5

Non-column standard:- 0.9ml of 0.1N phosphate buffer pH6.5 + 0.1ml of adrenaline or noradrenaline standard solution.

Spectrophotofluorimetric assay

The samples are assayed in batches of up to 15; the limiting factor being availability of the glass columns, (36 required for 15 samples plus standards). The tubes are arranged in the autoanalyser in the following order:-

TUBE NO.

1 + 2	Non-column blank.
3 + 4	Non-column adrenaline standard.
5 + 6	Non-column noradrenaline standard.
7 + 8	Column blank.
9 + 10	Column adrenaline standard.
11 + 12	Column noradrenaline standard.
13 -> 42	Samples 1 -> 15 in duplicate.

To all tubes at fixed time points the following reagents are added and mixed:-

TIME (minutes)	VOLUME (mls)	REAGENT
0	0.5	Potassium ferricyanide (0.05%) containing cupric chloride (0.00025%)
3	0.5	Sodium sulphite (10%) containing dimercaptopropanol (0.4%)
3.5	0.5	Sodium hydroxide (8N)
6.5	0.35	Glacial acetic acid.

Final pH = 5.0

At 30 minutes the sample is transferred to the spectrophotofluorimeter and the fluorescence read. The fluorescence is stable for more than 3 hours.

Excitation spectrum scanned over the range 300-500mμ

Emission wavelength 510mμ.

Notes on Catecholamine Assay

(1) Centrifugation of blood samples

It is known that loss of catecholamine occurs rapidly in whole blood (Mangan and Mason (1958)) but not in plasma (Jones and Blake (1958)). For this reason samples are kept on ice and are centrifuged at $4^{o}C$ within 6 minutes of collection.

(2) Omission of protein precipitation

Häggendal (1963) omitted protein precipitation from his assay and claimed he could run up to 10mls of plasma through Dowex 50W-X8 ion exchange resin (length 3.5cm, diameter 0.27cm) without affecting recovery.

Using Amberlite CG50 (length 5.0cm, diameter 0.25cm) it is not possible to use 'neat' plasma as column running times are in excess of 1 hour/ml. However, diluting the plasma greatly reduces running time. Plasma samples of 1ml (diluted to 4.5ml with phosphate buffer and E.D.T.A.) will run in 10-15 minutes.

(3) Removal of fibrin

The centrifugation to remove fibrin is not essential to the assay but, if omitted, will result in 'gumming-up' of the columns and very long running times will result.

(4) Volume of plasma used for extraction

Samples of more than 3mls of plasma are seldom collected. As duplicates are taken I have chosen a standard volume of 1ml. Using a constant volume reduces variation and keeps running times constant.

No reduction in recovery from the columns occurs using up to 4mls of plasma. However, if 4mls is exceeded recoveries fall. (see 'Choice of resin'). Samples of 1ml lie well within the efficient range of the columns.

(5) Choice of extraction technique

The two widely used techniques for catecholamine extraction are cation exchange and adsorption onto alumina. There is little to choose between the two techniques for recovery and reliability. However, the cation exchange technique is quick and simple whereas the preparation of alumina is long and tedious. Also, the adsorption of catecholamines onto alumina requires an alkaline pH which may increase the variability in recovery. Hence, cation exchange has been used.

(6) Choice of resin

The ideal resin should allow elution of the catecholamines in a volume of 1ml or less with recoveries in excess of 90%. Blanks should also be low and consistent.

Two resins, Amberlite CG50, a weak carboxylic acid resin, and Amberlite CG120, a strong sulphonic acid resin are compared.

Amberlite CG120

Haggendal (1966) demonstrated that catecholamine loss is a function of column length, i.e. the shorter the column the lower the recovery. However, on the other hand, the shorter the column the higher the flow and smaller volumes are required for elution. A suitable column length of Amberlite CG120 which gives recoveries consistently above 90% and adequate flow rates is 2cms.

To elute catecholamines from this strong sulphonic acid resin either a small volume of concentrated acid (1ml of 4N HCl) or a large volume of a weaker acid (4ml of 1N HCl or 10ml of 2/3M Boric acid) must be used. However, if 4N HCl is used high and variable blanks result. Therefore, the best alternative is to elute using 4mls of 1N HCl.

The catecholamines contained in this 4mls are further diluted when neutralised to pH6.5 using 5N potassium carbonate. As 1ml is the

ideal volume for the catecholamine assay less than a quarter of the sample is used, resulting in a loss of sensitivity.

Amberlite CG50

To obtain recoveries consistently above 90% a minimum column length of 3cms is required.

Catecholamines are eluted with ease from the weak carboxylic acid resin using 1ml of $^{2}/_{3}$M Boric acid. The 1ml eluted sample has a pH of 6.5 and no neutralisation is necessary. Therefore, sensitivity of the assay is maximum.

However, lower recoveries can result when the ionic content (or volume) of plasma is increased. To overcome this a 5cm column is used and the plasma volume fixed at 1ml. (see 'Volume of plasma used for extraction').

The reduction in flow which results from increasing column length from 3 to 5cms is small and of no concern.

If large plasma volumes were to be used, such as assay of peripheral catecholamines, the Amberlite CG120 would be the resin of choice. However, as plasma samples rarely exceed 3mls and duplicates are taken, Amberlite CG50 offers a more sensitive assay which is also simpler as two steps are omitted; neutralisation and pipetting of 1ml aliquots.

Therefore, for the assay of adrenal venous catecholamines, Amberlite CG50 has been used.

(7) Preparation of resin

Fluorescent contaminants are present in the resin but are removed by careful preparation.

Preparation is by the method of Renzini et al (1970) and is as follows:

a) Soak 10g Amberlite CG50 (100-200 mesh) overnight in 200mls of 2N HCl.
b) Wash several times with distilled water to remove fines (2 minutes sedimentation time).
c) Stir for 30 minutes in 200mls of 2N NaOH with a glass paddle stirrer (Magnetic stirring bars are not suitable as they produce too much grinding).
d) Wash 8-10 times with distilled water.
e) Stir for 30 minutes in 200mls of 2N HCl.
f) Wash 8-10 times with distilled water.
g) Repeat cycle c)-f) three times.
h) Transfer the resin to a glass column (4cm i.d.) and wash with 0.4M disodium phosphate buffer pH6.5 until the pH of the effluent is 6.5.
i) Store the resin in the same buffer at 2-4^{o}C.

j) Rinse the resin 3-4 times with distilled water before loading into columns.

Recycling of the resin between the expanded sodium form and the contracted hydrogen form expels impurities. Renzini claims the resin will keep for up to 4 months in buffer at 2-4^{o}C but as I used large quantities of resin none was kept for longer than 2 months.

(8) Column type

The columns are glass and consist of a 20ml reservoir above a narrow bore tube (0.25cm i.d.). A quartz wool plug at the end of the narrow bore tube retains the 5cm column of resin.

The prepared column is rinsed continuously with distilled water until the sample is ready for loading.

(9) Rinsing

As the resin is weak there is a danger that an excess of ions will wash catecholamines off the column. The columns are therefore rinsed using 5mls of distilled, deionised water. This does not increase blank values and ensures the catecholamines remain on the column.

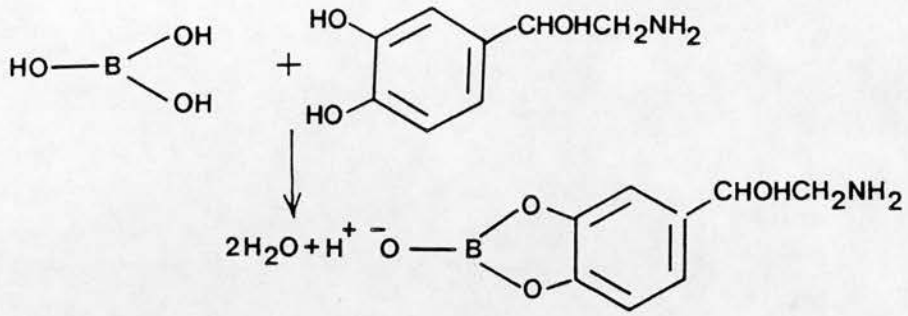
Following the water rinse, 0.15ml of $^{2}/_{3}$M Boric acid is loaded onto the column. This does not cause release of any catecholamine from the column but reduces the dead space the 1ml eluant will have to fill before elution begins. Omitting to add the 0.15ml of $^{2}/_{3}$M Boric acid does not alter recoveries but does affect the pH of the eluted sample such that a small volume of acid must be added to reduce the pH to the required 6.5.

(10) Elution

Catecholamines are eluted using 1ml of $^{2}/_{3}$M Boric acid. A volume of 1ml is used as this is adequate for virtually complete elution of catecholamines from the columns and is ideal for use in the tri-hydroxyindole fluorimetric assay. $^{2}/_{3}$M is the concentration chosen as boric acid is insoluble at room temperature at higher concentrations.

Trauther and Messer (1952) reported that adrenaline and noradrenaline form complexes with boric acid which promotes elution. The complexes are also highly stable at pH 7-8, making boric acid ideal for storing catecholamines prior to the fluorimetric assay.

Formation of borate-noradrenaline complex



Borate complexes have been used previously in the extraction of catecholamines and methods are described by Wright (1958), Mattock, Wilson and Heacock (1966), Sandhu and Freed (1968) and Renzini et al (1970).

Spectrophotofluorimetric assay

(11) Oxidation pH

A pH of 6.5 was used as, at this pH, the derivatives of adrenaline and noradrenaline have about equal fluorescence and variations of 0.5 pH units make little difference to the final fluorescence.

(12) Duplicates

Duplicates of all samples are taken here as they should be in any assay. This ensures reliability and provides two chances to obtain results from the assay should any misfortune occur.

(13) Blanks and standards

a) Blanks

Blanks are essential to quantify and allow for background fluorescence. Background fluorescence results from either the reagents themselves or any contaminants which may be present in the reagents or not removed from the sample during the purification procedure.

Two types of sample, or column, blanks are commonly used:- Non-oxidised blanks, where the antioxidant is added before the oxidant, and faded blanks, where the antioxidant is not added until complete oxidation of the catecholamines and their lutines to non-fluorescent derivatives has occurred.

Whilst these are the best blanks available to use for the assay of circulating catecholamines, they are not ideal, i.e. the samples and sample blanks are not treated identically and, as a consequence of this, errors may result in assessing the 'true' value of the blank. - As I am assaying catecholamines released from the adrenal medulla, and not circulating catecholamines, the true level is calculated thus:- Catecholamines released = Catecholamines in adrenal venous sample - Catecholamines in arterial sample. The assay used is not sensitive enough to measure peripheral catecholamine levels but a more accurate measure of

adrenal release may be made by using an arterial sample as the sample, or column, blank; treating it identically to any adrenal venous sample. This makes allowance for the catecholamines present which are not released by the adrenal medulla and also avoids any possible error which may arise through treating samples and blanks differently. An additional advantage in using this type of blank is that all tubes are processed in the same way, simplifying the assay and reducing the likelihood of operator error.

A second type of blank, the non-column or reagent blank, is also used. This is simply phosphate buffer and is used only in the standardisation of the spectrophotofluorimetric assay and not for the calculation of sample fluorescence.

b) Standards

As with blanks, two types of standard are taken. The first, column or plasma standards, are used for the calculation of adrenaline and noradrenaline in the adrenal venous samples. The second, non-column standards, to provide a measure of recovery from the columns. Adjustment to correct catecholamine release for recovery may be made but as all recoveries lay within 95-100% this was never done.

(14) Timing

Accurate timing is essential for all stages of the fluorimetric procedure. This was achieved simply and reliably by using an automated reagent delivery system, the 'Unicam AC60 autoanalyser'. Using the autoanalyser increases batch handling capacity from about 12 to 120. (120 is the number of sample holders in the autoanalyser). However, batches larger than 42 were never run owing to a limited number of columns being available.

(15) Potassium ferrycyanide

Potassium ferricyanide and iodine are widely used for the oxidation of catecholamines. Iodine is slower to oxidise catecholamines and is reported to be influenced by laboratory lighting conditions (Callingham (1967)). On the other hand, potassium ferricyanide yields more fluorescence from adrenaline and noradrenaline than oxidation with iodine and, at the same time, reduces interference from dopamine and dopa (at the wavelengths used). (Weil-Malherbe (1971), Valori et al (1970)). Therefore potassium ferricyanide is used here.

A solution of potassium ferricyanide is stable for up to one month if kept refridgerated but to ensure reliability it is made up daily as required.

(16) Cupric chloride

Häggendal (1963) was first to use cupric ions during the oxidation of catecholamines, claiming they catalysed the oxidation process and increased the stability, reproducibility and intensity of fluorescence. Valori et al (1970) confirmed the catalytic effect of cupric ions but mainly in relation to the oxidation of adrenaline. Valori et al (1970) also noted that cupric ions increased the fluorescent intensity of adrenolutine, even when added after the reducing agent or alkalinization; indicating that cupric ions act during both the oxidation reaction and the rearrangement of the adrenochrome to adrenolutine.

As it is accepted that metal ions (e.g. Cu^{2+} and Zn^{2+}) play a part in the oxidisation of catecholamines and their derivatives I have added cupric chloride to the sample, prior to oxidation. Rather than add the cupric chloride and potassium ferricyanide separately the procedure is simplified by mixing the two reagents and dispensing them as one solution.

(17) Sodium sulphite/dimercaptopropanol (B.A.L.)

A mixture of BAL and sodium sulphite is used to stop the oxidation and stabilize the strongly fluorescent trihydroxyindole derivatives, adrenolutine and noradrenolutine.

Ascorbic acid was originally used as the reducing agent by Lund (1949) and by most workers for the following 10 years. However, since Vendsalü (1960) noted that ascorbic acid in alkali gives rise to fluorescent oxidation products, which produce high and time dependent blank values, various attempts have been made to replace ascorbic acid by other reducing agents, all of them containing sulphur, e.g. BAL/sodium sulphite mixture (Häggendal (1963)), thioglycolic acid (Merrills (1963)) and cysteine hydrochloride (Klensch (1966)). The BAL/sodium sulphite mixture gave the most satisfactory results, maintaining the high lutine fluorescence seen with ascorbic acid but yielding smaller and more reproducible blanks.

The solubility of BAL in sodium sulphite is poor and clear solutions are difficult to obtain. However, if the solution is vigorously shaken and only used when fresh (not kept for more than 1 hour) problems such as variable blanks and quenching, reported by Valori et al (1970), do not occur.

Valori et al (1970) attempted to overcome the problem by dissovling BAL at higher concentrations (removing the need for other antioxidants) in solutions which increase the solubility of BAL in water. - They describe a technique in which a solution of 10% BAL in 25% formaldehyde is used. However, this produces poor fluorescence and is not as satisfactory as using the original BAL/sodium sulphite mixture.

(18) Glacial acetic acid

All samples are reacidified to pH 5.0 by the addition of 0.35mls of glacial acetic acid.

The stability of fluorescence formed from both adrenaline and noradrenaline in the presence of a sulfhydryl compound is greatly enhanced by reacidification. Authors using ascorbic acid as the reducing agent (Chang (1964) and Price and Price (1957)) found reacidification did not offer any advantage. Laverty and Taylor (1967), whilst studying the optimal conditions for the development of fluorescence, found that oxidation products formed from adrenaline and noradrenaline by iodine could be sufficiently stabilized with a solution of sodium sulphite alone, if followed by acidification. Oxidation with ferricyanide yields more fluorescence from adrenaline and noradrenaline than oxidation with iodine; but omission of BAL from the sodium sulphite solution results in losses of intensity and stability of fluorescence.

In our experience reacidification results in a shift of the fluorescence spectrum which is maximum at pH 5.0. The first peak for both catecholamines is greatly reduced whilst the second peak is increased. The adrenaline fluorescence is increased preferentially with the ratio:- adrenaline peak height: noradrenaline peak height, changing from about 0.8 to 1.2 (at emission wavelength 510mμ). Acidification thereby increases the discrimination of the assay and at the same time prevents fading of the fluorescence.

The fluorescence is stable for at least 3 hours at room temperature and is little affected by a pH change of 0.5 units in either direction.

(19) Reading of fluorescence

23.5 minutes following the addition of glacial acetic acid the sample is transferred from the autoanalyser to the spectrophotofluorimeter, the outside of the tube being carefully wiped to remove any contamination. The emission wavelength is set to 510mμ and the excitation spectrum scanned from 300mμ to scatter, at about 500mμ.

The intensity of the fluorescence is displayed on the linear scale of an Aminco Bowman Photomultiplier Microphotometer and also on a 'Servoscribe' flat-bed recorder. The chart drive of the recorder is synchronised to the scanning motor switch on the spectrophotofluorimeter; thus, the chart drive only operates when a scan is in progress. As the chart and wavelength scanning motor speed are fixed, and never varied, the wavelength at any position on the fluorescence trace can be found by its distance from the start of the scan (123 millimetres is equivalent to 100mμ), i.e. - excitation wavelength = 300 + (distance in millimetres from start/123 x 100)mμ.

To minimise the error in reading fluorescence intensity the meter multiplier switch on the photomultiplier, and the sensitivity switch, on the flat-bad recorder, are adjusted to give the maximum reading possible. The scales on both the meter multiplier and the servoscribe sensitivity were checked and found to be true and linear.

The fluorescence of the standards, adrenaline and noradrenaline, were superimposed for easy assessment of the degree of differentiation.

(20) Selection of wavelengths

The fluorimetric assay has to fulfil two requirements,

1. to provide a sensitive assay for the measurement of catecholamines and
2. to differentiate between adrenaline and noradrenaline.

The discriminative power of the assay can be quantified using the method of Gaddum (1959), replacing 'activity' with fluorescence intensity; i.e.:-

$$\text{I.O.D.} = \frac{\text{F.I. of Adrenaline/F.I. of Noradrenaline (at the higher wavelength)}}{\text{F.I. of Adrenaline/F.I. of Noradrenaline (at the lower wavelength)}}$$

where I.O.D. is index of discrimination and F.I. is fluorescence intensity.

Examples of excitation scans at emission wavelengths 500, 510 and 520mμ are given (Figs. 4, 5 and 6). As the emission wavelength is increased from 500 to 520mμ the index of discrimination increases but the fluorescence is reduced, (especially fluorescence due to noradrenaline), thereby decreasing the sensitivity of the assay. The sensitivity is maximum at 500mμ where the fluorescence of adrenaline and noradrenaline are equivalent. However, a differential assay at this wavelength is poor and unreliable as the scatter, which occurs at lower excitation wavelengths, can interfere; particularly when catecholamine levels are low. An emission wavelength of 510mμ is a compromise between sensitivity and discrimination and has been used here except in several early 'selective release' experiments where 520mμ was used. In these experiments catecholamine levels were high in any case and the ratio, noradrenaline : adrenaline was the major parameter sought.

375 and 450 mμ were selected as the best excitation wavelengths to use in the differential assay of adrenaline and noradrenaline. The separation of the two fluorescence traces is maximum at these two points and interference from scatter and the first, almost non-existent, peak does not occur. Fluorescence intensity is also significantly greater than the blank value at these wavelengths.

Figure 4 Reduced photocopy of fluorescence spectra of 100 ng of adrenaline and noradrenaline

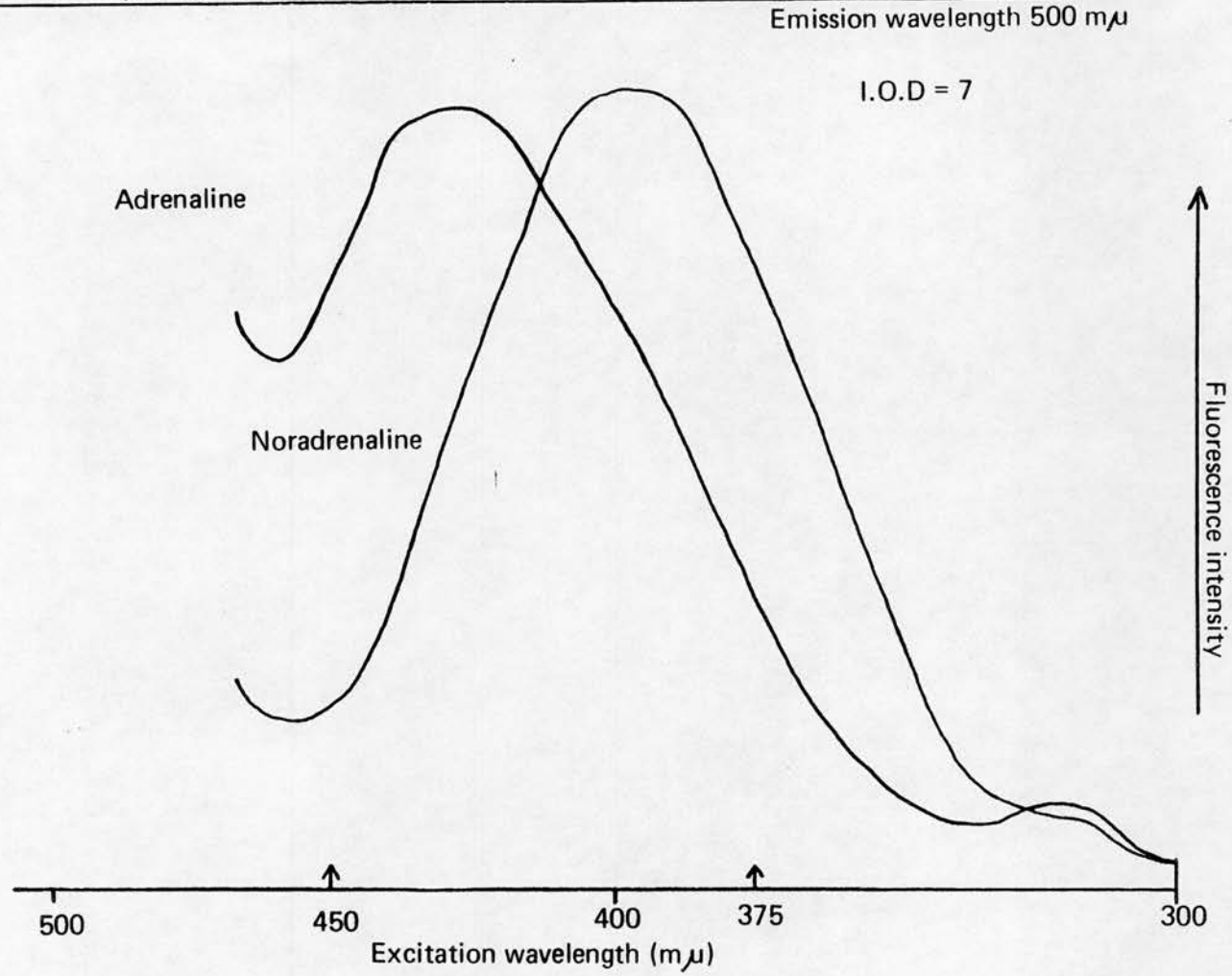


Figure 5. Reduced photocopy of fluorescence spectra of 100 ng of adrenaline and noradrenaline

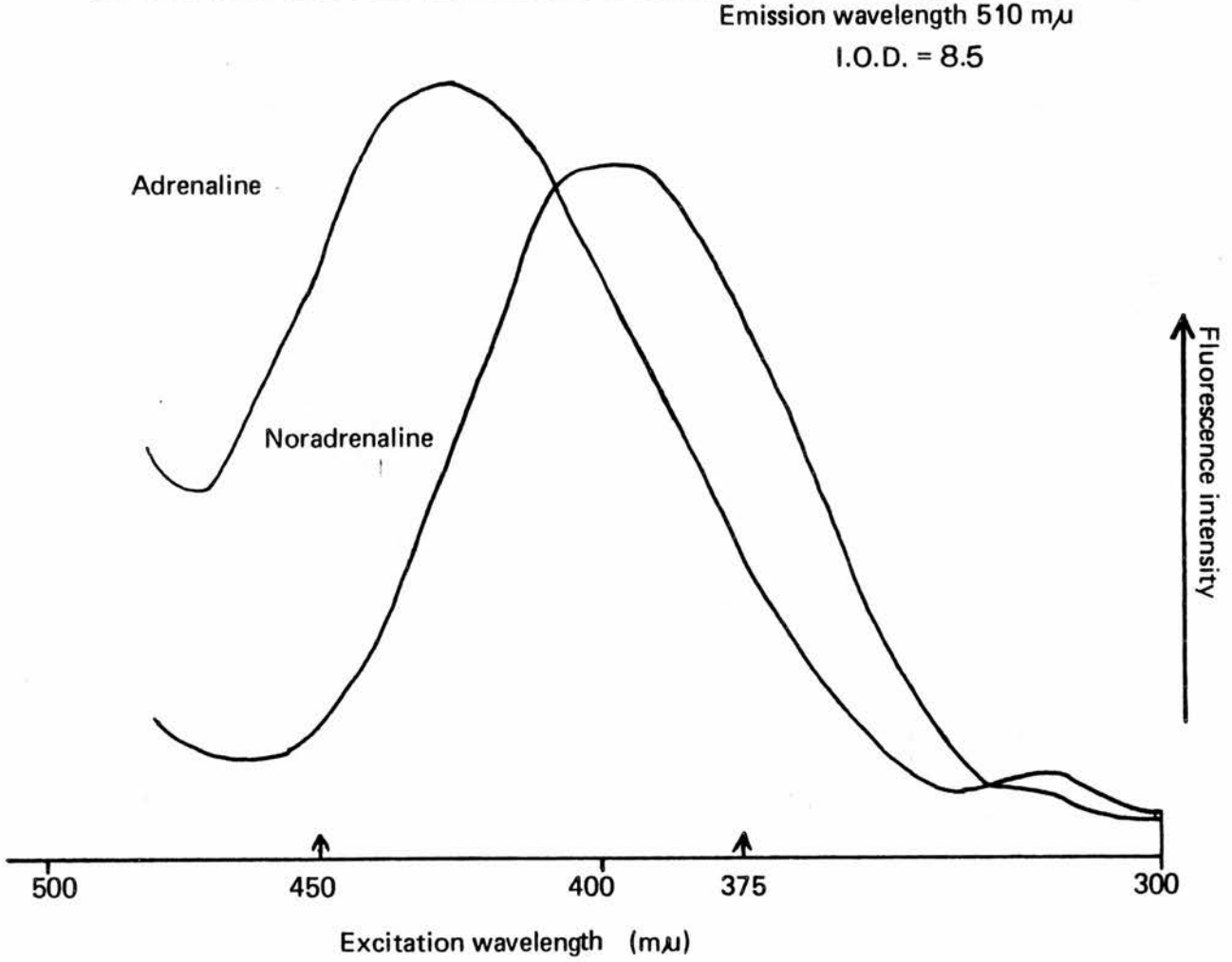
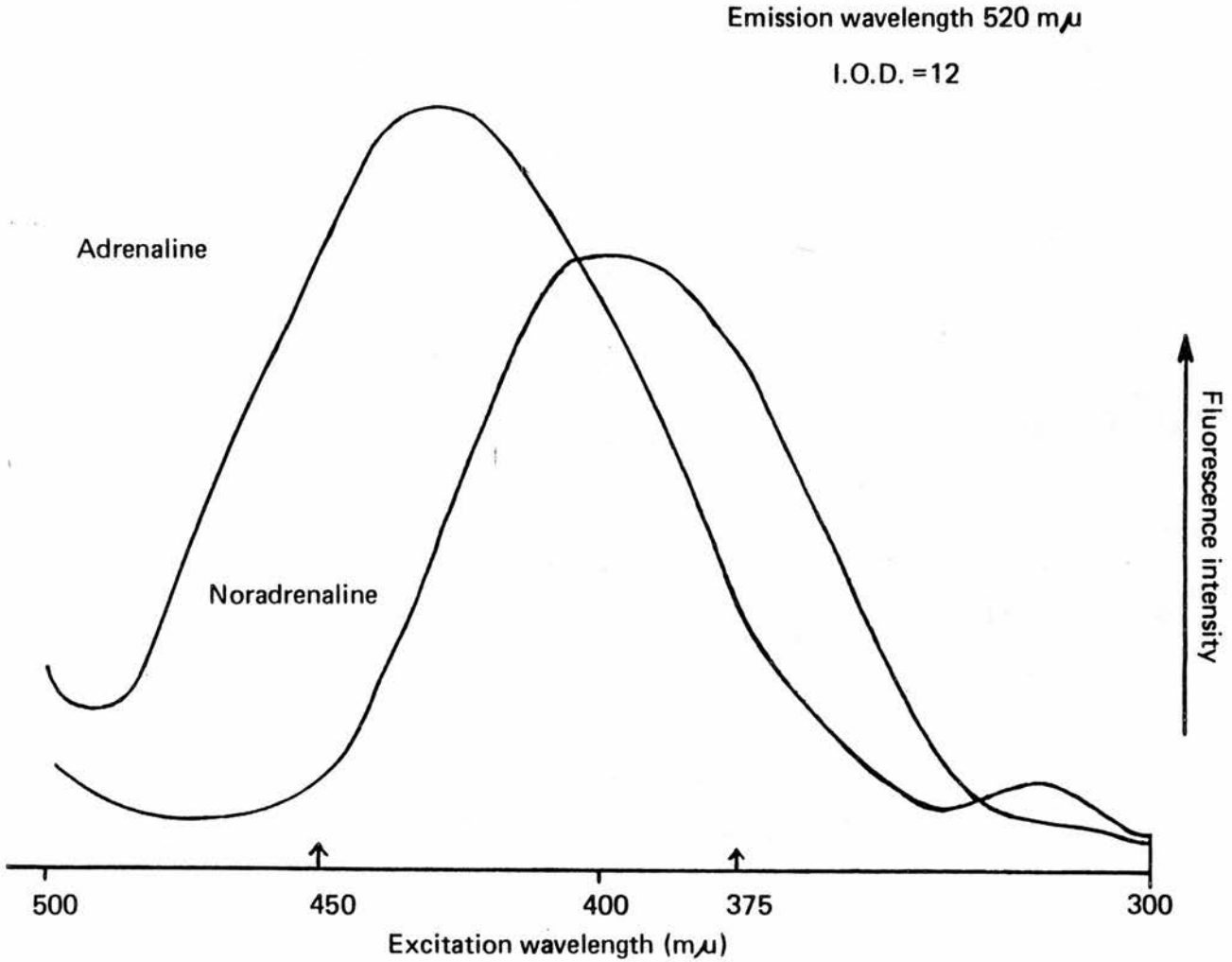


Figure 6 Reduced photocopy of fluorescence spectra of 100 ng adrenaline and noradrenaline



(21) Calculation of adrenaline and noradrenaline

Assuming the relationship between fluorescence intensity and concentration is linear and the fluorescence of the two catecholamines is additive an equation can be written which describes the fluorescence at any given point on the spectra.

$$\text{i.e. } F = yN + xA,$$

where F is the fluorescence intensity, N and A are the fluorescnece intensities per nanogram of noradrenaline and adrenaline respectively and y and x are the quantities of noradrenaline and adrenaline in the sample.

If fluorescence readings are taken at two wavelengths, 375 and 450mμ, then a pair of simultaneous equations can be written,

$$\text{i.e. } F_1 = yN_1 + xA_1 \quad \text{and} \quad F_2 = yN_2 + xA_2,$$

where 1 and 2 refer to 375 and 450mμ respectively. Solving these equations yields,

$$y = \frac{F_1(A_2/A_1)-F_2}{N_1(A_2/A_1)-N_2} \quad \text{and} \quad x = \frac{F_2-yN_2}{A_2}$$

As I usually use 100ng standards of adrenaline and noradrenaline y and x must be multiplied by 100 to give the amounts of noradrenaline and adrenaline present in the sample.

A simple program for use with a 'Hewlett Packard' calculator was written to solve these equations when analysing samples.

The two assumptions on which these equations are based have been tested by other authors and found valid. I have tested them myself, using the assay procedure previously described, by assaying known concentrations of adrenaline and noradrenaline and also equivalent mixtures of the two catecholamines. The results are displayed in figure 7.

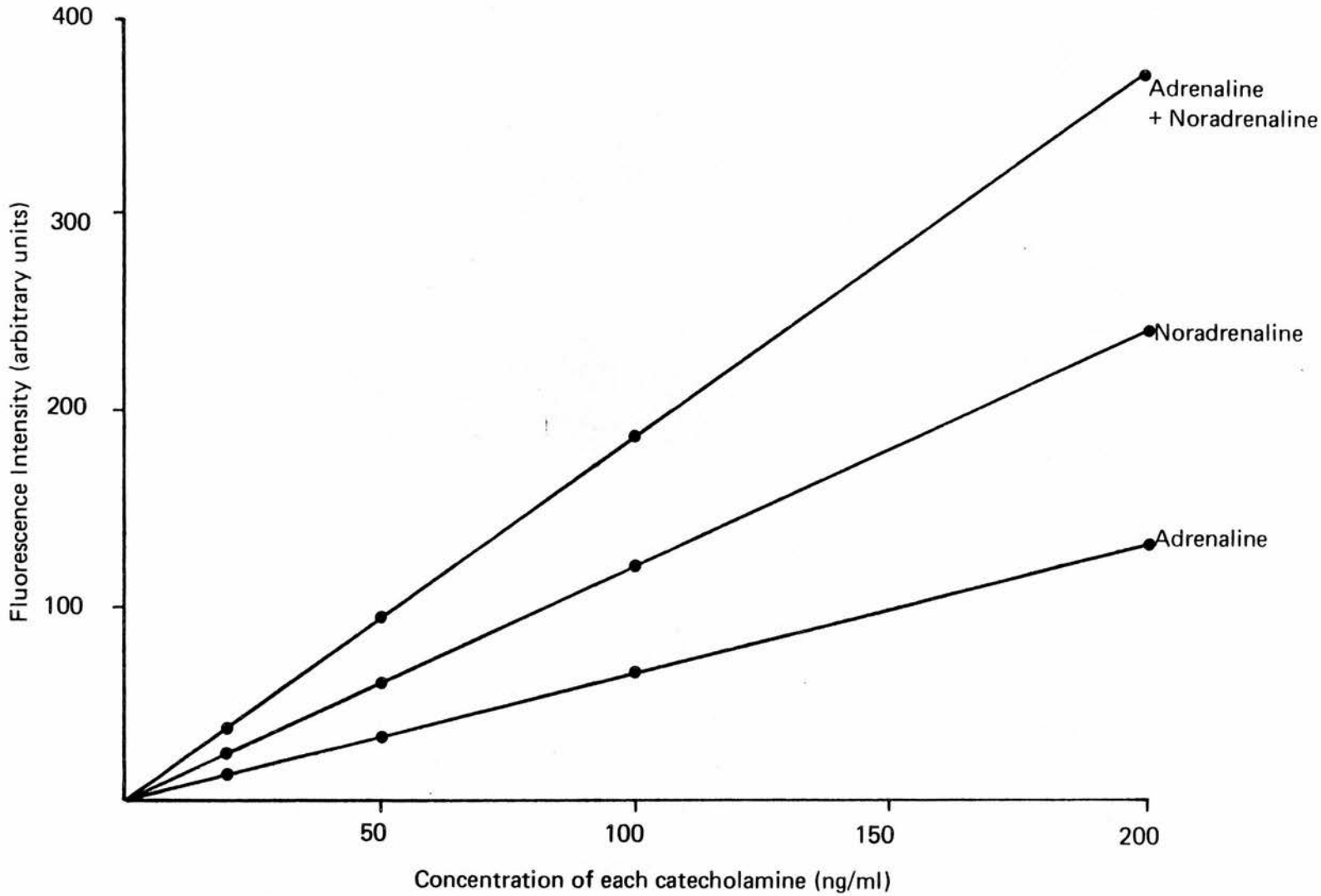
A linear relationship between fluorescence intensity and concentration exists and fluorescence is additive. i.e. The assumptions are valid for this assay. The results shown are taken at only one wavelength, 375mμ, but the same relationship holds true at any wavelength.

(22) Discrimination of the assay

The assay was tested for its ability to discriminate between adrenaline and noradrenaline by assaying known mixtures of standards.

In the examples given the emission wavelength is 510mμ.

Figure 7 Calibration curves of adrenaline and noradrenaline, fluorescence read at 375/510 mμ



MIXTURE			CALCULATED (Mean ± S.E.)	
n	A	NA	A	NA
4	100	0	) Used as standards	
4	0	100	)	
4	90	10	90.5 ± 0.6	8.1 ± 1.2
4	80	20	79.4 ± 0.7	17.4 ± 1.4
4	70	30	71.2 ± 1.1	27.0 ± 1.6
4	60	40	60.1 ± 0.8	38.3 ± 0.6
4	50	50	52.6 ± 1.2	49.5 ± 1.2
4	40	60	40.9 ± 1.0	57.1 ± 1.5
4	30	70	32.1 ± 1.0	67.8 ± 1.6
4	20	80	18.2 ± 1.1	79.0 ± 1.1
4	10	90	9.0 ± 1.5	88.2 ± 0.8

METHODS

Whole Animal Experiments

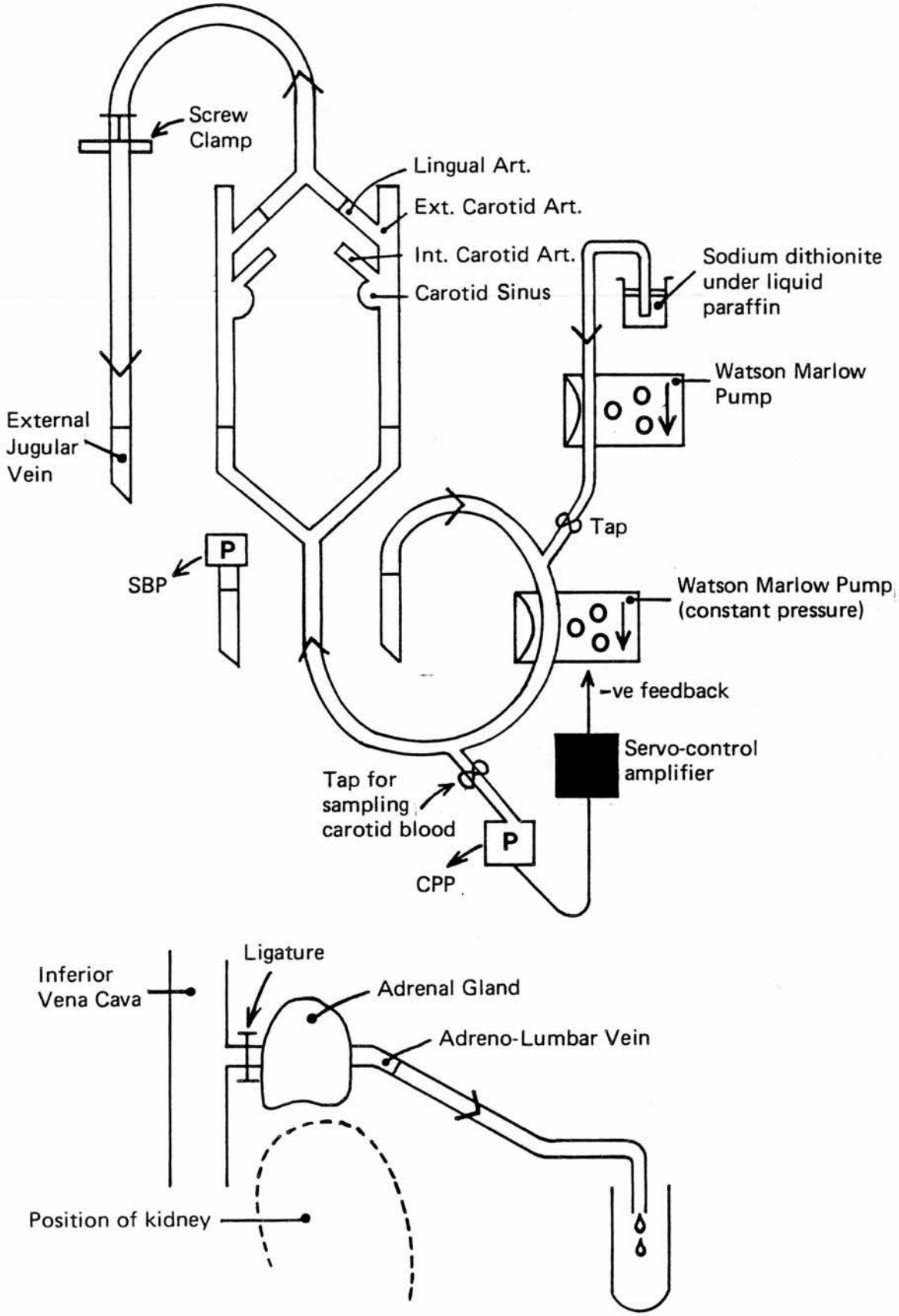
Outline of Technique

The experimental preparation used to investigate the reflex and humoral release of adrenaline and noradrenaline from the adrenal medulla is illustrated in figure 8.

The preparation is an anaesthetised dog in which both carotid bifurcations are vascularly isolated and perfused, at constant pressure, with the dog's own arterial blood. The release of catecholamines is stimulated by lowering the carotid sinus pressure (barotest) or, alternatively, by lowering the oxygen tension of the blood perfusing the carotid bifurcations by infusing the reducing agent sodium dithionite (chemotest).

The venous effluent from the left adrenal gland is collected and later assayed for adrenaline and noradrenaline by the trihydroxyindole spectrophotofluorimetric technique.

Figure 8 Diagram of perfusion of vascularly isolated carotid bifurcations and collection of adrenal venous blood samples.



Details of Method

Anaesthesia

Dogs were anaesthetised with an i.v. injection of either sodium pentobarbitone (30mg/kg) or chloralose (55mg/kg) and urethane (550mg/kg). When using chloralose-urethane the dogs were premedicated half an hour before induction with morphine sulphate (1mg/kg) dissolved in 0.9% saline and given sub-cutaneously. The premedication allowed the large volume of chloralose-urethane necessary for anaesthesia to be injected with ease and the minimum of trauma.

Anaesthesia was maintained by a continuous i.v. infusion of anaesthetic at a rate adjusted to just supress the paw withdrawal reflex, - about 10% of the induction dose per hour.

Pentobarbitone anaesthesia was used in only five experiments and was employed solely to obtain results which could be compared to existing data obtained in this laboratory from cats under pentobarbitone anaesthesia. The use of pentobarbitone was not continued as I found it selectively depressed the chemoreceptor reflex relative to the baroreceptor reflex release of catecholamines in the dog. Chloralose-urethane anaesthesia was used in all other experiments as it has little reflex depressant activity (Heymans and Neil, (1958)) and is commonly used in the study of cardiovascular reflexes.

Some adrenal depleting activity has been reported for several anaesthetics, particularly those incorporating morphine and administered to cats, (Vogt, (1954), Emmelin and Strömblad, (1952), Outschoorn, (1952) and Elliot, (1912)). As morphine has a stimulant action in the cat but not the dog, the morphine premedication was not considered a problem. In any case, adrenal collections were not started for at least four hours following premedication by which time any effect of the low dose of morphine (1mg/kg compared to about 20mg/kg necessary for depletion in the cat) used would be negligible. Chloralose-urethane per se is not known to cause adrenal depletion.

Respiration, Acid-Base Balance and Temperature Control

The trachea was cannulated and connected to a Starling 'Ideal' pump. The lungs were ventilated with a metered oxygen-nitrogen mixture, the mixture adjusted to maintain $PaCO_2$ at 39mmHg and PaO_2 above 150mmHg, measured from arterial blood samples on a Radiometer BMS 3 analyser. Any base deficit in the arterial plasma was corrected by injecting the appropriate volume of sodium bicarbonate (1 molar), according to the Singer-Hastings nomogram (1948).

The respiration rate was fixed at 25 per minute and the stroke volume adjusted with reference to the arterial blood gas tensions and particularly the ventilatory efforts of the dog.

Body temperature was held at 37°C by a heating pad and lamp linked to a rectal thermistor probe.

Maintenance of Systemic Arterial Blood Pressure

Systemic arterial blood pressure was constantly monitored and maintained by an i.v. infusion of Dextran, prewarmed to 37°C.

Systemic arterial blood pressure falls as a result of blood loss through bleeding and sample collection, prostaglandins released by surgical trauma (for details see under 'Prostaglandins and adrenal blood flow') and bladder reflexes resulting from a distended bladder (e.g. Taylor, (1968)).

To reduce the quantity of Dextran necessary to maintain systemic arterial blood pressure the bladder was catheterised and drained and in some experiments indomethacin (5mg/kg) was given prior to abdominal surgery to prevent any possible prostaglandin release.

Carotid Perfusion

Both common carotid and lingual arteries were surgically exposed and prepared for cannulation.

The carotid bifurcations were then prepared for vascular isolation by placing loose ligatures around all branches between the points of cannulation on the common carotids and the origins of the lingual arteries. The branches include the superior thyroid, internal carotid, external carotid, occipital and ascending pharyngeal arteries and occasionally other variable and non-identified branches.

The superior thyroid arteries are tied off immediately and the common carotid arteries cannulated both ways; blood from one common carotid perfusing both towards the head via a Watson Marlow MHRE pump. The cannula from the second common carotid artery is connected to a pressure transducer for the measurement of systemic blood pressure.

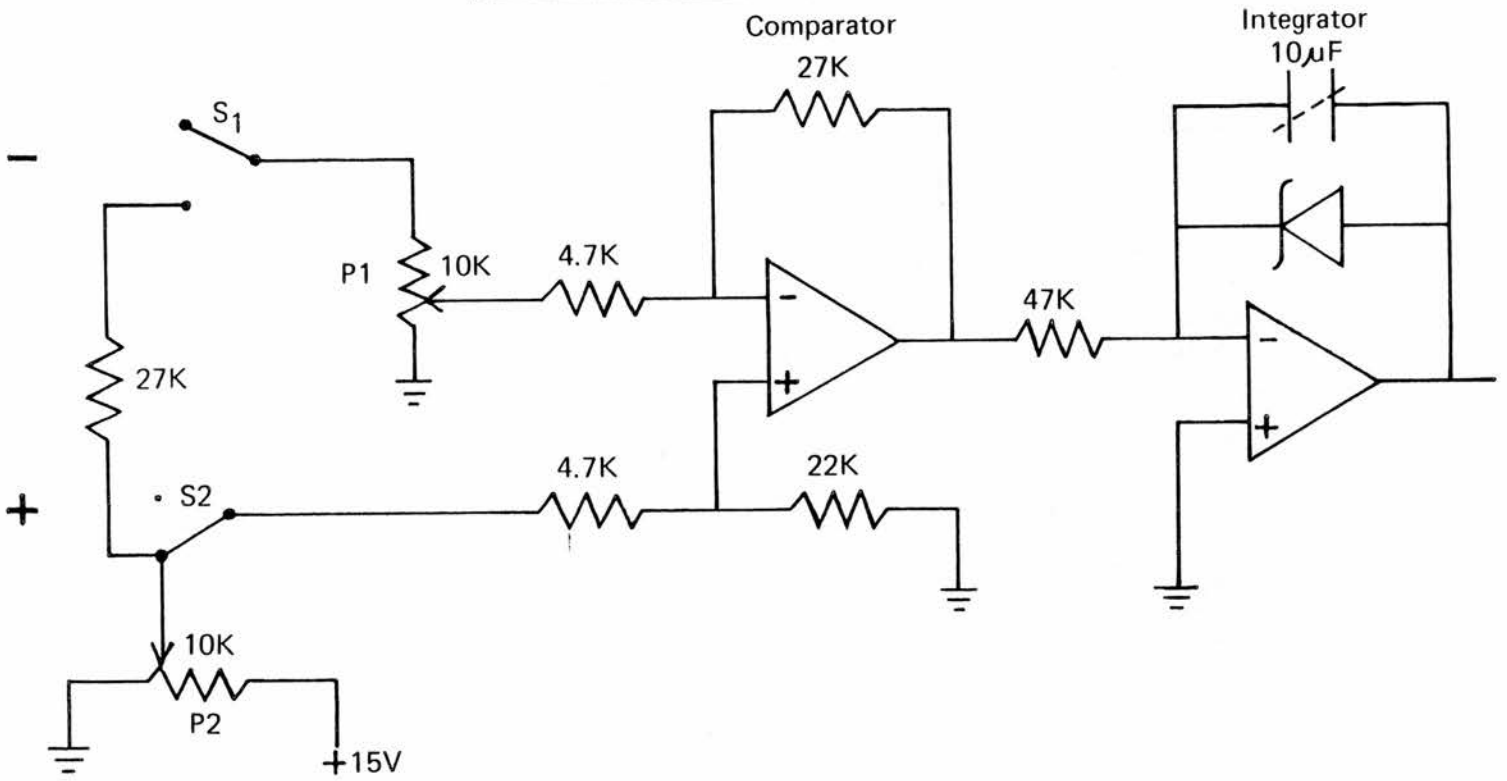
Using a Y-shaped catheter with a cannula connected to each fork both lingual arteries are cannulated towards the heart and the blood returned to the animal via the right external jugular (see figure 8).

All loose ligatures are then tied, vascularly isolating both carotid bifurcations.

The lingual arteries are not ligated but cannulated to maintain blood flow through the system and thus allow changes in the perfusing blood to affect the carotid bodies quickly. The resistance is adjusted by a screw clamp placed around the cannula returning blood to the external jugular.

A pressure transducer connected to the perfusion circuit, measuring carotid perfusion pressure (CPP), was linked, through a servo-amplifier to the Watson Marlow perfusion pump. The servo control system (see figure 9 for circuit diagram) works by negative feedback to control the speed of the pump, maintaining a constant perfusion pressure which is set by a 'clock' potentiometer. Thus, carotid perfusion pressure may be

Figure 9 Circuit diagram for Servo Control Amplifier System



varied by simply turning the potentiometer switch which has been calibrated in millimetres of mercury (mmHg).

To test the vascular isolation the Watson Marlow perfusion pump should stop when the flow through the lingual arteries is occluded.

In several of the earlier experiments the lingual arteries were not cannulated but simply left open, to allow an adequate flow of blood through the carotid bifurcations. In this instance we had assumed that anastomosis between the lingual artery and the brain was negligible. This assumption was questioned, so, to remove any possibility of carotid blood reaching the brain, the lingual arteries were cannulated and the blood diverted. No differences were detectable between the two techniques.

Stimulation of Reflexes

a) Baroreceptor tests

Baroreceptor tests are a lowering of the carotid sinus pressure, produced by lowering the setting on the clock potentiometer by 40mmHg for 60 seconds. This reduces stimulation of the baroreceptors which initiates the baroreceptor reflex.

A high arterial PaO_2 is maintained throughout such that chemoreceptor stimulation in response to the fall in carotid perfusion pressure is minimal.

b) Chemoreceptor tests

Chemoreceptor tests are a lowering of the PaO_2 of the blood perfusing the carotid bifurcations, at constant pressure, produced by infusing into it a 1 molar solution of sodium dithionite, (Critchley and Ungar, (1975)). By altering the rate of infusion graded hypoxic stimuli can be given down to a PaO_2 of zero.

Frequent blood gas samples are taken to monitor the degree of hypoxia, hypercapnia and acidaemia achieved during each test.

Systemic arterial blood gas tension does not alter when sodium dithionite is infused into the carotid circuit.

Two types of chemoreceptor test are performed:-

1. Short duration (CR), where sodium dithionite is infused for only 90 seconds and the PaO_2 reduced to about 25-30mmHg, and

2. Long duration (LCR), where sodium dithionite is infused for up to 20 minutes and the PaO_2 is only reduced to about 45mmHg.

Both vagosympathetic trunks are cut in the neck to abolish any secondary reflexes from thoracic receptors.

Figure 10 Example of barotest and chemotest showing effects on systemic blood pressure and carotid perfusion pressure

Examples of the effects of baroreceptor and chemoreceptor tests on carotid perfusion pressure and systemic blood pressure are shown in figure 10.

Collection of Adrenal Venous Blood Samples

In the dog the venous effluent from the adrenal gland drains into the adreno-lumbar vein which then runs only a short distance until it fuses with either the inferior vena cava or the renal vein.

It is impractical to cannulate the short length of vein between the adrenal gland and the inferior vena cava, therefore, the adreno-lumbar vein is cannulated retrogradely to enable collection of adrenal venous blood.

The adreno-lumbar vein is cleaned and prepared for cannulation a short distance from the adrenal gland. All tributaries between the gland and the point of cannulation are tied off. A loose ligature is placed around the adrenal vein between the adrenal gland and the inferior vena cava.

Cannulation of the adreno-lumbar vein is the final step in the setting-up procedure and is not performed until after heparinisation and the completion of the carotid perfusion circuit.

Soft, flexible polythene tubing, with as wide a bore as possible, is used to cannulate the adreno-lumbar vein. Once the tubing is tied in position the adrenal vein distal to the adrenal gland is ligated. Adrenal venous blood then flows retrogradely via the cannula and is either collected in cooled graduated centrifuge tubes or collected in a reservoir from which blood is returned to the animal at a constant infusion rate via the left femoral vein. This minimises blood loss.

Adrenal venous blood samples were usually collected over a 2 minute period; but if adrenal blood flow was either very high or very low the collection time was adjusted to provide a sample volume of between 2 and 10mls.

In all experiments adrenal venous blood has been collected from the left adrenal gland. The right adrenal gland lies more superior than the left and is virtually inaccessible for cannulation.

Denervation of the left adrenal gland

The left splanchnic nerve was identified and cut about 3cms from the adrenal gland. The retroperitoneal connective tissue was dissected in an arc around the gland to interrupt additional sympathetic fibres, particularly around the arteries supplying the gland. The abolition of the adrenal response to baroreceptor tests, while the pressor response remained, was taken as evidence of complete denervation.

This technique of surgically denervating the adrenal gland is traumatic and produces bleeding owing to the high vascularity of the area. In an attempt to reduce the trauma two dogs were treated with lignocaine injections around the adrenal gland and distinct nerve fibres and blood vessels were crushed between the tips of non-serrated artery forceps. This produced a temporary but totally complete block of catecholamine release from the adrenal gland.

This technique was abandoned as lignocaine appears to penetrate the adrenal gland and act directly on it to prevent even basal release of catecholamines.

Our success rate for denervating the left adrenal gland was as low as 55%.

Surgical Protocol

1. Cannulate trachea.
2. Cannulate hind limb vein for anaesthetic infusion.
3. Prepare carotid bifurcations for vascular isolation and perfusion.
4. Cut vagi in the neck.
5. Administer indomethacin (5mg/kg) - if to be given.
6. Open abdomen (Laparotomy).
7. Put animal on respirator.
8. Prepare left adreno-lumbar vein for cannulation.
9. Allow 30 minutes for haemostasis.
10. Administer intravenous heparin (500 i.u./kg).

11. Set up carotid perfusion and isolate bifurcations.

12. Check blood gases and adjust acid-base balance.

13. Cannulate adreno-lumbar vein and commence adrenal venous collection.

Experimental Protocol

a) Reflex and Selective Release Experiments (Dogs 1-17, and 34)

A detailed protocol for each experiment is contained in Appendix 2, 'Tables of Results from Whole Animal Experiments'.

An outline of the general protocol used for all reflex and selective release experiments is presented here:-

TIME (in minutes)	ACTION
0	Start control sample collection.
2	Stop control sample collection. Barotest on (Lower CPP by 40mmHg).
~2.5	Start barotest sample collection (see note 1).
3	Barotest off.
~4.5	Stop barotest sample collection.
9	Start control sample collection.
11	Stop control sample collection. Chemotest on (Infuse sodium dithionite).
~11.5	Start chemotest sample collection (see note 2).
12.5	Chemotest off.
~13.5	Stop chemotest sample collection.

The cycle of baroreceptor and chemoreceptor tests is repeated at about 20 minute intervals. The drugs, hexamethonium bromide and hyoscine methyl bromide, are normally administered after every two cycles.

Samples of blood perfusing the carotid bifurcations are taken during control collections and during periods of chemoreceptor stimulation (chemotest) for blood-gas analysis and pH determination.

Note 1 Barotest sample collections are started approximately half a minute after the start of reflex stimulation in order to allow for the dead space of the collecting system. The time delay is calculated for each collection from knowing the dead space (mls) and dividing it by the adrenal blood flow (mls/min), assumed to be the same as the preceeding control period.

The sample is collected for 2 minutes.

Note 2 During chemotests allowance is made for the dead space in the collecting system as described in note 1. However, as there is also a delay between starting the sodium dithionite infusion and initiation of the chemoreceptor reflex, the dead space allowance is made from the time of the first forced inspiratory movement of the dog, which is

assumed to coincide with stimulation of the chemoreceptors. The chemotest sample is then collected for two minutes.

Occasionally a second chemotest collection is taken immediately following the first, (CR′). This was taken to ensure that the peak release of catecholamines was not being missed.

b) Neural and Endocrine Mechanisms of Catecholamine Release (Dogs 18-33)

A detailed protocol for each experiment is contained in Appendix 2, 'Tables of Results from Whole Animal Experiments'.

This study is comprised of 5 groups of experiments. Briefly, they are:-

1. Effects of Exogenous ACTH.
   (Dog nos. 18, 19 and 21-26).
   Synacthen, 25 or 250µg, is infused intravenously. Adrenal venous collections are taken before, during and at timed intervals after synacthen infusion.

2. Effects of Long Duration Chemotests (LCR).
   (Dog nos. 18, 19, 22, 23, 26, 32 and 33).
   Adrenal venous collections are taken before, during and at

timed intervals after chemotests of either 10 or 20 minutes duration. (see 'Stimulation of Reflexes').

3. Effects of LCR in dogs with denervated left adrenal glands. (Dog nos. 27-31).
   Protocol as 2.

4. Effects of LCR in dogs treated with cycloheximide (50mg/kg). (Dog no. 32).
   Protocol as 2.

5. Effects of LCR in dogs with denervated left adrenal glands and treated with cycloheximide (50mg/kg).
   (Dog no. 31).
   Protocol as 2.

Samples of blood are taken frequently from the carotid circuit to monitor the degree of hypoxia during long chemotests. A PaO_2 of 45mmHg was aimed for during long chemotests.

All adrenal sample collections were taken over two minutes.

c) Prostaglandins and Adrenal Blood Flow

Most of the information relating prostaglandins and adrenal blood flow is obtained by comparing two groups of dogs, those given indomethacin and those not.

However, one experiment was performed in which the adrenal venous blood was assayed for PGI_2 or, more accurately the metabolite of PGI_2, PG-6-OXO-$F_{1\alpha}$.

The protocol is similar to that used for 'Reflex and Selective Release Experiments' except that only barotests were performed and indomethacin was the major drug administered rather than hexamethonium bromide or hyoscine methyl bromide.

For a detailed protocol see Dog no. 20, Appendix 2.

The following parameters were recorded in all experiments and are presented in Appendix 2:

Arterial blood gas tensions and pH, systemic blood pressure, carotid sinus pressure, adrenal blood flow and catecholamine release as adrenaline and noradrenaline. In addition, time from the start of the experiment and the dog's sex and weight are recorded.

Isolated Adrenal Gland Perfusion Experiments

Introduction

There is conflicting evidence over the effects of indomethacin and prostaglandins on adrenal catecholamine secretion; the controversies probably resulting from species differences and diverse investigative methods.

From the results presented herein for whole animal experiments, (Appendix 2), indomethacin appears to inhibit catecholamine secretion both at rest and during baroreceptor tests. As systemic blood pressure and adrenal blood flow are altered by indomethacin it is impossible to say with certainty that the reduction in catecholamine secretion is a direct effect of indomethacin and not simply a reflex response to increased blood flow and systemic blood pressure.

To investigate the effects of indomethacin and prostaglandins on catecholamine secretion by the adrenal medulla, without interference from changes in pressure and flow, indomethacin and prostaglandins were administered to isolated perfused canine adrenal glands.

Source of Glands

Adrenal glands were removed from dogs immediately after death. The donors were all dogs which had been used by the Department of Medicine, University of Edinburgh, for the investigation of myocardial damage

following coronary occlusion and had not received any adrenal stimulants. Unfortunately glands could not be removed prior to death as removal of the heart marked the end point of the experiment. However, if the glands are rapidly removed and immediately flushed with heparinised Locke's solution, the formation of blood clots is prevented and the resistance to perfusion minimised - If clots have formed the gland swells and ruptures when flushed.

Removal of Glands

The adrenal glands are cannulated in situ in a retrograde fashion via the adrenolumbar vein, using a short length of polythene tubing with a tightly fitting luer needle, with the point removed, fixed in one end. The adrenal gland is dissected free from the retroperitoneal tissue surrounding it and the adrenal vein cut beyond the ligature. The gland is removed attached to the cannula and is immediately flushed with heparinised Locke's solution. The gland is stored in Locke's solution until used some 30 minutes later.

Perfusion

The adrenal glands are perfused with phosphate buffered Locke's solution (NaCl 154mM, Glucose 10mM, KCl 5.6mM, Na_2HPO_4 2.15mM, $NaH_2PO_4.2H_2O$ 0.85mM, $CaCl_2.6H_2O$ 2.2mM) at a constant flow of 2mls/minute, delivered by a Watson Marlow MHRE pump. The Locke's solution is continuously oxygenated and maintained at 37°C by a heated water bath and passage through a heated coil. A pump circulates water from the heated bath to the coil and also to a 'Langendorff' jacket which surrounds the gland.

The gland is suspended in a small glass funnel and effluent from the gland drips directly from the funnel into collecting tubes held in the conveyor belt of the Unicam AC60 Autoanalyser. The belt of the autoanalyser moves every 30 seconds and thus 1ml samples of adrenal effluent are collected.

Drug Infusions

To ensure equilibration the gland was perfused for a minimum of 60 minutes before drugs were administered.

All drugs were infused for 2.5 minutes and samples collected before, during and after drug infusion according to the following protocol:-

TIME (mins)	DRUG INFUSION	COLLECTION
0		CONTROL
0.5		-
1		CONTROL
1.5	———>START	CONTROL
2		-
2.5		-
3		-
3.5		TEST
4	———>STOP	TEST
4.5		TEST
5		TEST
5.5		TEST
6		-
6.5		-
7		RECOVERY
7.5		-
8		-
8.5		RECOVERY
9		-
9.5		-
10		RECOVERY

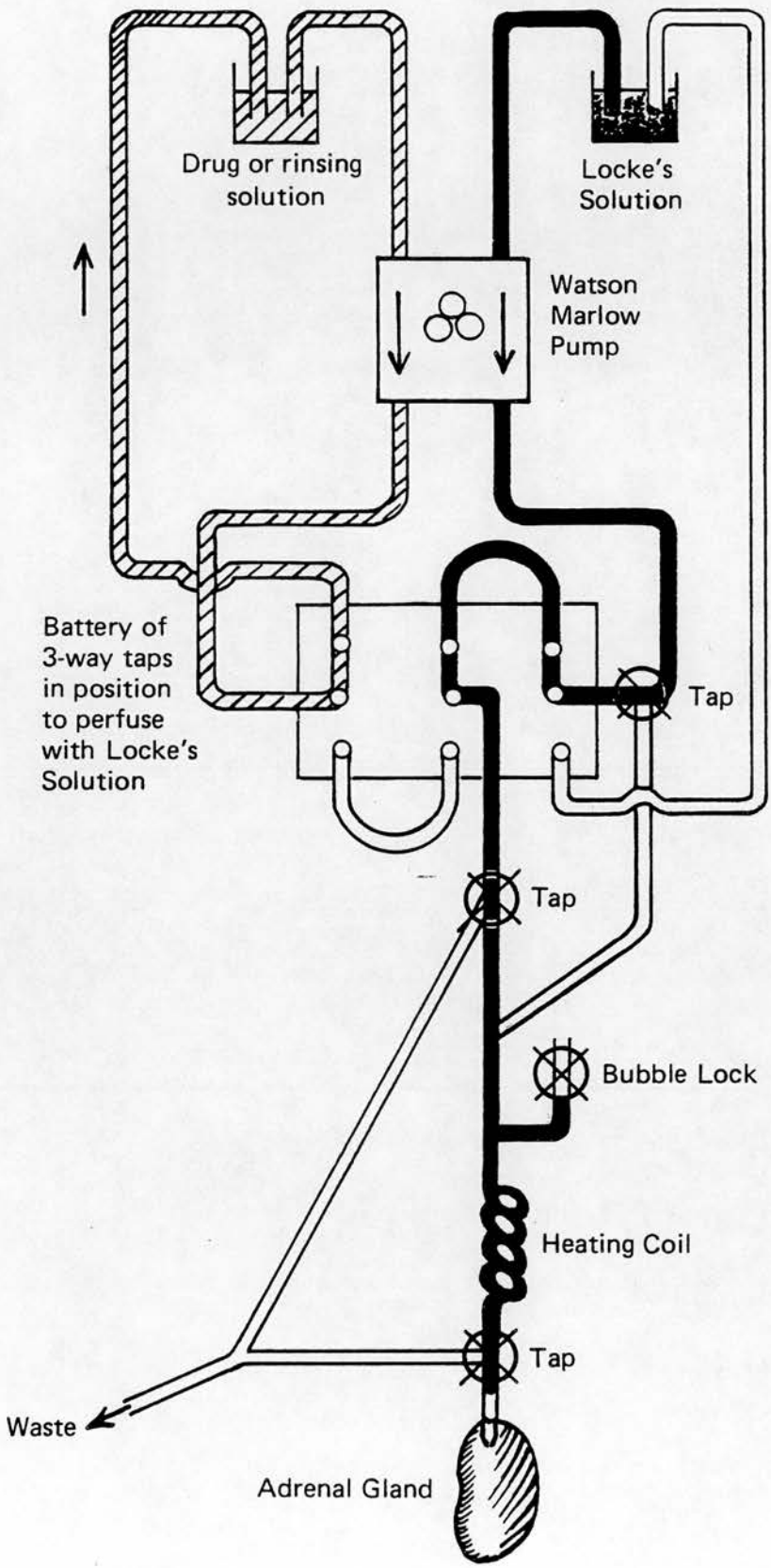
The test collections do not coincide with drug infusion times as the dead space is 4mls and 2 minutes are required for the drug containing perfusate to reach the adrenal gland.

All drugs were made up fresh for each test in Locke's solution, oxygenated and heated to 37°C in a water bath prior to and during each infusion. Stock prostaglandin E_2 was dissolved in ethanol but was diluted many-fold in Locke's solution to give the desired concentrations for use here - A blank of ethanol in Locke's solution produced no change in catecholamine release from an isolated adrenal gland.

Drug solutions and Locke's perfusate were pumped simultaneously at 2mls/minute by one double carriage Watson Marlow pump, one solution directed to the adrenal gland and the other recycled. This ensures no change in flow occurs when the perfusate is changed from Locke's solution to Locke's containing drug and vice versa. The changeover of perfusate is made rapidly and smoothly by a single level action which operates a series of 3-way taps interconnected by a system of short tubes (see figure 11). This mechanism ensures that no interruption in flow occurs during changeover. The drug solution remaining in the system at the end of a test period is completely washed out with Locke's solution without interfering with the perfusion of the adrenal gland.

In all experiments control collections were taken before and after changeover to confirm that the changeover procedure itself did not produce any release of catecholamine from the adrenal gland.

Figure 11 Diagram of isolated adrenal gland perfusion system



At least 30 minutes were allowed between drug infusions for catecholamine secretion to return to a steady state. This also allowed sufficient time to complete the spectrophotofluorimetric assay of adrenaline and noradrenaline in the samples collected before starting another run.

Individual experimental protocols of drug infusions are listed in Appendix 3, 'Tables of results from isolated adrenal gland experiments'.

Collection of Samples

The moving track of the Unicam AC60 Autoanalyser was used as a fraction collector for the adrenal effluent, moving every 30 seconds and thus collecting samples of 1ml.

The adrenal gland was suspended in its heating jacket and funnel above the track of the AC60 autoanalyser. A short piece of portex tubing attached to the spout of the funnel is positioned to just touch the rims of the collecting tubes passing beneath it. This prevented any loss of effluent as the tubes moved and, by collecting part drops, ensured the same volume of effluent was collected in each sample.

A waste pipe and funnel was positioned beneath the collecting point to catch the adrenal effluent between collections.

Catecholamine Estimation

As the track of the AC60 Autoanalyser moved, sample tubes passing the collection point were removed, advanced along the track and replaced in order behind tubes containing either Locke's solution only (blanks) or standards of adrenaline and noradrenaline made up in Locke's solution, i.e.

Tube No.

1 + 2 - Reagent blanks, 1ml of Locke's solution.

3 + 4 - Adrenaline standards, 100ng adrenaline in 1ml of Locke's solution.

5 + 6 - Noradrenaline standards, 100ng noradrenaline in 1ml of Locke's solution.

7 - Adrenal sample control [used as faded blank].

8 - 17 - Adrenal samples

The tubes are positioned immediately before the first reagent dispensing station on the autoanalyser; a metal pin being placed alongside the first tube and one space behind the last tube to switch the dispensing stations on and off respectively.

The tubes pass the four dispensing points and the reagents for the trihydroxyindole spectrophotofluorimetric assay are added.

The trihydroxyindole method used here is identical to the method used for the assay of adrenaline and noradrenaline in adrenal venous samples collected from whole animals and a full account of the method giving times, reagent volumes, reading of fluorescence, etc. is already presented, see 'Experimental Protocol for Catecholamine Assay - Spectrophotofluorimetric Assay'.

Blanks

Reagent blanks of 1ml of Locke's solution were used in determining the fluorescence produced by the adrenaline and noradrenaline standards.

A faded blank, in which the BAL/sodium sulphite mixture was not added until 15 minutes after the other reagents, was used in the calculation of catecholamines contained in adrenal samples. A faded blank was used rather than the reagent blank to correct for any fluorescent contaminants released from the adrenal gland. However, the difference between the blanks was negligible indicating very little contamination.

Standards

Tubes containing 1ml of Locke's solution were prepared and incorporated into the previously described sequence. Just before the oxidising reagent is added 20µl of either adrenaline or noradrenaline (5µg/ml) is added and the solution mixed.

Stock solutions of adrenaline and noradrenaline (5µg/ml) are made up using 10^{-3}M HCl.

Calculation of Adrenaline and Noradrenaline

The calculation of adrenaline and noradrenaline in the samples is carried out using the method previously described. (Notes on Catecholamine Assay - Calculation of Adrenaline and Noradrenaline). The peak release occurring during drug perfusion is recorded and expressed as ng/minute.

Comments on the Isolated Adrenal Gland Perfusion System

Perfusion of the adrenal glands by the method I have described is an accepted and established technique. However, the technique is hardly 'physiological' as the adrenal gland is perfused retrogradely and with Locke's solution, not blood.

To overcome these two problems the adrenal gland must be perfused in situ, via the aorta, with the animal's own blood - This technique is expensive, requiring the use of a whole animal and, as our resources were limited, we did not attempt it.

An alternative technique is reported, (Nahus, (1970)), which describes the retrograde perfusion of isolated adrenal glands with dilute homologous blood. This technique is undoubtedly more physiological but does require sacrificing animals for blood and, in addition, would necessitate the inclusion of an extraction procedure prior to the spectrophotofluorimetric assay. This would markedly reduce the throughput of the assay to only two drug infusions per gland.

Therefore, whilst aware of the limitations of the artificial perfusion technique described, it has been used here to clarify and provide support for results obtained from whole animals.

Results and Discussion

A. Selective Release of Adrenaline and Noradrenaline from the canine adrenal gland

Results

We have studied the release of adrenaline and noradrenaline evoked by the baroreceptor and chemoreceptor reflex from the canine adrenal medulla, under a variety of different conditions. These include differing anaesthesia, pentobarbitone or chloralose-urethane; the presence or absence of the nicotinic and muscarinic antagonists, hexamethonium bromide and (-)hyoscine methyl bromide, the prostaglandin synthetase inhibitor indomethacin, and also cycloheximide, an inhibitor of the release of corticosteroids in response to adrenocorticotrophic hormone. In addition, catecholamine release has been investigated from the adrenal medulla of dogs with denervated adrenal glands.

The results are summarised in table 1, which shows the percentage of noradrenaline released under all circumstances.

TABLE 1

Percentage Noradrenaline in Adrenal Venous Samples (Mean ± S.E.)

TREATMENT OR DRUG / TEST	NONE	HEXA-METHONIUM	HYOSCINE	HEXA-METHONIUM & HYOSCINE	DENERVATION	CYCLO-HEXIMIDE
A. Pentobarbitone anaesthesia						
CONTROL	17 ± 3 (n=18)	39 ± 7 (n=6)	15 ± 4 (n=6)	18 ± 8 (n=2)	-	-
BAROTEST	20 ± 3 (n=9)	37 ± 6 (n=3)	11 ± 2 (n=3)	33 (n=1)	-	-
CHEMOTEST	16 ± 2 (n=9)	31 ± 5 (n=3)	9 ± 1 (n=2)	40 (n=1)	-	-
B. Chloralose/Urethane anaesthesia						
CONTROL	23 ± 2 (n=41)	34 ± 13 (n=4)	15 ± 2 (n=4)	17 ± 6 (n=2)	24 ± 4 (n=9)	24 ± 1 (n=3)
BAROTEST	25 ± 3 (n=23)	25 ± 5 (n=2)	14 ± 4 (n=2)	12 (n=1)	26 ± 5 (n=7)	24 ± 2 (n=2)
CHEMOTEST	21 ± 5 (n=7)	16 ± 7 (n=2)	17 ± 2 (n=2)	10 (n=1)	-	-
LONG CHEMOTEST	24 ± 5 (n=5)	-	-	-	21 ± 2 (n=3)	32 (n=1)
ACTH	20 ± 5 (n=4)	-	11 (n=1)	-	-	-
C. Chloralose/Urethane anaesthesia + Indomethacin (5mg/kg)						
CONTROL	22 ± 3 (n=42)	58 ± 18 (n=2)	26 ± 5 (n=17)	-	-	-
BAROTEST	26 ± 5 (n=25)	75 (n=1)	31 ± 10 (n=9)	-	-	-
CHEMOTEST	32 ± 6 (n=17)	59 ± 14 (n=3)	21 ± 5 (n=8)	-	-	-

No significant differences in the ratio of noradrenaline:adrenaline were detected during either control periods, stimulation of baroreceptor or chemoreceptor reflexes or the infusion of Synacthen (ACTH). In addition, the ratio of noradrenaline:adrenaline is independent of the anaesthetic used, an intact nerve supply and the presence of the drugs cycloheximide, indomethacin and (-)hyoscine methyl bromide.

The only evidence of selective release occurring 'in vivo' is a small but consistent increase in the resting secretion of noradrenaline following treatment with the nicotinic antagonist hexamethonium bromide.

No evidence for selective release of either noradrenaline or adrenaline was found on investigating catecholamine release from the isolated canine adrenal gland; nicotine or prostaglandin E_2 being used to evoke release in the presence or absence of hexamethonium bromide or indomethacin. Two glands, numbers 2 and 3, appeared to selectively release noradrenaline when stimulated with nicotine. However, this was not supported in other glands where nicotine either did not alter the noradrenaline:adrenaline ratio or even reduced it.

The overall mean percentage of noradrenaline released 'in vivo' is 24 ± 1 ($n = 313$, S.D. = 18). If the results in the presence of hexamethonium bromide are discounted then the mean percentage falls only slightly, and non-significantly, to 23%.

The mean percentage noradrenaline release 'in vitro' agrees closely with this and is 22 ± 1 ($n = 116$, S.D. = 15).

Discussion

Many authors have argued for and against the independent control of the release of adrenaline and noradrenaline. If one considers only work where catecholamines are estimated in adrenal venous blood, and not peripheral blood, then studies on the cat provide the bulk of evidence in support of independent control (von Euler and Folkow (1953), Folkow and von Euler (1954), Düner (1953)) whereas studies in the dog, in the main, do not support independent control. (Malmejac (1964), Critchley (1976)).

To investigate a possible species difference Critchley and Ungar (Critchley (1976)) studied the release of adrenaline and noradrenaline from the adrenal medulla of both cats and dogs using similar techniques of stimulation, collection and assay. As evidence existed in cats suggesting that stimulation of the baroreceptor reflex preferentially released noradrenaline and stimulation of the chemoreceptor reflex preferentially released adrenaline, (Anichkov *et al* (1960)), Critchley and Ungar used these two stimuli to test for independent control of adrenaline and noradrenaline release. Their results clearly demonstrated independent control of release in the cat, supporting the conclusions of Anichkov *et al*(1960).The ratio noradrenaline:adrenaline rising from 1:1 at rest to 3:1 in the increment over control during baroreceptor tests and falling from 1:1 to 1:6 during chemoreceptor tests. In dogs the ratio remained fixed at 1:4 both at rest and during reflex stimulation, in agreement with

Malmejac (1964), De Schaepdryver (1959) and Wurtman, Casper, Pohorecky and Bartler (1968) who also failed to find any evidence for selective release from the canine adrenal medulla in response to physiological stimulation.

Although Crithley and Ungar employed similar techniques of reflex stimulation, adrenal venous collection and catecholamine assay for studies on both dogs and cats they did not use the same method of anaesthesia. Cats were anaesthetised with pentobarbitone and dogs with a mixture of chloralose and urethane. As it is well established that anaesthetics can effect catecholamine release from the adrenal medulla, (see `Methods, Whole animal experiments and DeSchaepdryver (1959)), Dr. Ungar and I repeated the study in dogs under both chloralose-urethane and pentobarbitone anaesthesia. The results, as summarised in table 1, clearly show no evidence for independent control of adrenaline and noradrenaline release by the baroreceptor or chemoreceptor reflex under either chloralose-urethane or pentobarbitone anaesthesia. The percentage of noradrenaline released remains fixed at about 20-25% which is in agreement with figures quoted by other workers. Therefore, the difference demonstrated between cats and dogs is not due to differences in anaesthesia. The collected results are published (Critchley, Ellis and Ungar (1980)) and the paper is attached in the back of this thesis.

Through studying the reflex release of catecholamines from dogs under pentobarbitone anaesthesia we were surprised to find the adreno-medullary response to the chemoreceptor reflex was markedly inhibited, (in all but one dog), relative to the baroreceptor reflex in spite of

strong respiratory and vascular responses. The opposite, depression of the baroreceptor response relative to the chemoreceptor response, has been reported for cats under chloralose anaesthesia (Neil, Redwood and Schweitzer, (1949)). This reinforces the need for careful consideration before selecting an anaesthetic especially for use in reflex experiments. It is for this reason that we have used chloralose-urethane anaesthesia in the dog for all adrenomedullary reflex experiments, apart from the one particular study where pentobarbitone was used for comparative purposes.

Although in agreement that the baroreceptor and chemoreceptor reflexes do not selectively release adrenaline or noradrenaline I have presented data which suggests the nicotinic antagonist, hexamethonium bromide, increases the percentage of noradrenaline in the resting release. More accurately, as hexamethonium reduces the basal secretion of catecholamines from the adrenal medulla, it selectively reduces the release of adrenaline. As the canine adrenal medulla contains both muscarinic and nicotinic receptors which mediate catecholamine release, (Kayaalp and Türker (1969)) this would suggest that stimulation of the muscarinic receptors would selectively increase noradrenaline release.

Similar findings have been reported by others. Kayaalp and McIsaac (1968) have reported a selective increase in noradrenaline release with methacholine (10-20μg/kg), a muscarinic receptor stimulant, as compared to dimethylphenylpiperazinium iodide, (3-5μg.kg^{-1}), a nicotinic receptor stimulant. In addition, Kayaalp and McIsaac (1968) also found acetylcholine selectively released noradrenaline in the presence of the nicotinic

antagonist mecamylamine. However, acetylcholine was also reported to increase noradrenaline release in the presence of the muscarinic antagonist atropine. De Schaepdryver (1959), who reported no selective release following carotid occlusion, also measured catecholamine release stimulated by acetylcholine in the presence of atropine. He detected a substantial reduction in the percentage of noradrenaline released following pretreatment with atropine (40% before atropine and 20% after). De Schaepdryver (1959) also claimed low doses of nicotine (0.1mg/kg) reduced the percentage of noradrenaline released. However, this change was small, 37 to 29 percent, and as the dose of nicotine was increased the percentage of noradrenaline released was also increased e.g. 1.5mg/kg nicotine increased the percentage of noradrenaline from 32 to 38 percent.

Apart from experiments involving nicotinic and muscarinic agonists and antagonists, selective release has been reported which is dependent on the frequency of stimulation of the splanchnic nerve. Increasing frequency caused increased release of noradrenaline. (Rapela and Covian (1954), Klepping (1956), Rapela (1956) and Malmejac et al (1957)).

Although this evidence indicates independent control of release of adrenaline and noradrenaline in the dog, the noradrenaline released never exceeds the adrenaline even when selective release of noradrenaline is occurring. Thus, the majority of the evidence supporting independent release in the dog, is based on small, and frequently non-significant alterations in the ratio of noradrenaline: adrenaline. The evidence for independent control of release of

adrenaline and noradrenaline from the cat, not only by the baroreceptor and chemoreceptor reflex (as previously discussed) but also by nicotinic and muscarinic stimulation (Douglas and Poisner (1965), Critchley (1976)) is much more convincing. On comparing cat with dog it would be easy to dismiss any variation in the noradrenaline:adrenaline ratio in the dog as small and probably insignificant. However, the resting release of noradrenaline:adrenaline in the dog is about 1:4 compared to 1:1 in the cat, such that any small change in the percentage of noradrenaline released in the dog may be of more physiological significance than it would be in the cat. If independent control of adrenaline and noradrenaline does occur in the dog then a study comprising large numbers under very controlled conditions may be necessary to confirm it. Also, if as suggested by the authors cited here, the release of noradrenaline from the canine adrenal medulla is under muscarinic control, then a different and opposite functional role of muscarinic and nicotinic receptors of the cat and the dog will have been identified.

B. Effects of Cholinergic Antagonists on the Reflex Release of Catecholamines from the canine adrenal gland

Results

In anaesthetised dogs the release of adrenaline and noradrenaline from the adrenal medulla was studied following stimulation of the carotid baroreceptor and chemoreceptor reflexes.

The action, on reflex catecholamine release, of the nicotinic antagonist, hexamethonium bromide, the muscarinic antagonist (-)hyoscine methyl bromide and both drugs combined was investigated.

Only dogs which produced matched pairs of baroreceptor and chemoreceptor tests were used to calculate the results. Any mismatched stimuli were disregarded (Marked * in tables 2-7).

The abbreviations used in the tables of results (tables 2-7) are:-

CPP = Carotid perfusion pressure.

C.A. = Catecholamine (Adrenaline + noradrenaline).

C_6 = Hexamethonium bromide.

-HMB = (-)Hyoscine methyl bromide.

BR = Baroreceptor test.

CR = Chemoreceptor test.

a) The action of hexamethonium bromide on the release of catecholamines from the adrenal medulla

In 7 dogs baroreceptor and chemoreceptor tests were performed before and between 15 and 80 minutes after the intravenous administration of the nicotinic antagonist hexamethonium bromide (10mg/kg). This dose is several times the minimum effective dose as 2mg/kg is equally effective in abolishing any pressor response due to baroreceptor tests. The high dose ensures effective block for at least 80 minutes and is still selective for nicotinic receptors.

As the systemic blood pressure falls after hexamethonium administration the drug was given along with an infusion of dextran to reduce the fall.

The results are displayed in tables 2 and 3.

Hexamethonium significantly inhibited the adrenal response to both baroreceptor and chemoreceptor tests. The inhibition was consistently greater during baroreceptor tests.

b) The action of (-)hyoscine methyl bromide on the release of catecholamines from the adrenal medulla

In 4 dogs baroreceptor and chemoreceptor tests were performed before and between 15 and 70 minutes after the intravenous

administration of the muscarinic antagonist (-)hyoscine methyl bromide (10mg/kg).

The results are displayed in tables 4 and 5.

(-)Hyoscine methyl bromide significantly inhibited the adrenal response to both baroreceptor and chemoreceptor tests. In two dogs the chemoreceptor response was more substantially inhibited and in one dog the baroreceptor response was more inhibited.

c) The combined action of hexamethonium bromide and (-)hyoscine methyl bromide on the release of catecholamines from the adrenal medulla

In 2 dogs there was no residual response to either baroreceptor or chemoreceptor tests in the presence of both antagonists (10mg/kg of each).

The results are displayed in tables 6 and 7.

The resting output of one dog was approximately zero and was much reduced in the other.

The complete results of this study are summarised graphically in figure 12.

TABLE 2

BAROTEST RELEASE BEFORE AND AFTER HEXAMETHONIUM

DOG NO.	BEFORE			AFTER			% INHIBITION OF C.A. RELEASE BY C_6
	TEST	CHANGE OF CPP (mm Hg)	C.A. RELEASE ($ng.kg^{-1}min.^{-1}$)	TEST	CHANGE OF CPP (mm Hg)	C.A. RELEASE ($ng.kg^{-1}min.^{-1}$)	
6	BR 1	132-99	9.12	BR 3	127-98	0.54	
	BR 2	129-99	16.32	BR 4	126-96	0.79	95%
9	BR 1	120-92	7.02	BR 3	118-82	-0.26	
	BR 2	120-93	13.79	BR 4	117-90	0.00	101%
11	BR 1	116-80	6.22*	BR 3	105-67	0.89	
	BR 2	105-69	0.90*	BR 4	105-68	0.84	86%
13	BR 1	118-83	3.04	BR 3	115-79	0.61	
	BR 2	118-83	6.16	BR 4	113-78	1.03	82%
14	BR 1	125-87	0.17	BR 3	124-85	-0.34	
	BR 2	125-86	1.88	BR 4	121-83	0.68	92%
				BR 5	121-84	-0.10	
15	BR 1	130-115	18.37	BR 3	130-90	3.42	
	BR 2	130-100	28.65	BR 4	130-90	-0.05	93%
17	BR 1	145-110	0.88	BR 3	146-110	0.19	
	BR 2	147-118	2.29	BR 4	147-112	0.06	92%
MEAN		127.3-95.8	8.76		123.0-87.5	0.55	94%
STD. ERROR		2.7 3.5	2.33		3.2 3.3	0.24	

TABLE 3

CHEMOTEST RELEASE BEFORE AND AFTER HEXAMETHONIUM

DOG NO.	BEFORE			AFTER			% INHIBITION OF C.A. RELEASE BY C_6
	TEST	CHANGE OF PO_2 (mmHg)	C.A. RELEASE ($ng.kg.^{-1}min.^{-1}$)	TEST	CHANGE OF PO_2 (mmHg)	C.A. RELEASE ($ng.kg.^{-1}min.^{-1}$)	
6	CR 1 CR 2	205-41 168-37	16.67 37.12	CR 3 CR 4	149-34 146-41	4.08 3.94	85%
9	CR 1 CR 2	109-36 142-3.5	-5.23* 1.20	CR 3 CR 4	177-0.5 187-14	0.80 0.16	60%
11	CR 1 CR 2	190-29 200-32	14.59 16.57	CR 3 CR 4	218-29 212-27	2.44 2.68	84%
13	CR 1 CR 2	208-41 235-15	3.02 16.63*	CR 3 CR 4	240-33 255-27	1.00 2.32	45%
14	CR 1 CR 2	230-44 210-32	15.99 13.07	CR 3 CR 4 CR 5	212-22.5 225-3.5 200-36.5	0.89 0.40* 1.10	93%
15*	CR 1 CR 2	198-24.5 190-2	5.26 -10.85	CR 3 CR 4	195-25 195-3	-1.36 1.66	NO VALUE CALCULABLE
17	CR 1 CR 2 CR 3	123-37 116-35 112-24.5	-0.51* -0.48* 0.9	CR 4 CR 5	114-23 111-27	0.32 0.51	54%
MEAN STD. ERROR		185-31.6 12.5 4.1	13.24 3.73		185.1-26.2 13.5 3.1	1.69 0.39	87%

TABLE 4

BAROTEST RELEASE BEFORE AND AFTER HYOSCINE METHYL BROMIDE

DOG NO.	BEFORE			AFTER			% INHIBITION OF C.A. RELEASE BY -HMB
	TEST	CHANGE OF CPP (mmHg)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)	TEST	CHANGE OF CPP (mmHg)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)	
7	BR 1	------	2.71	BR 4	122-83	2.13	
	BR 2	124-94	1.89	BR 5	122-83	1.52	
	BR 3	123-84	2.55				23%
10	BR 1	136-105	9.72	BR 3	136-101	1.83	
	BR 2	135-101	5.72	BR 4	135-103	2.06	75%
12	BR 1	123-87	5.61	BR 3	121-83	3.86	
	BR 2	123-90	4.44	BR 4	121-81	2.40	41%
				BR 5	119-83	2.93	
				BR 6	119-83	2.60	
16	BR 1	140-102	1.48	BR 4	141-116	1.28	
	BR 2	140-111	1.82	BR 5	141-112	0.86	58%
	BR 3	140-123	3.10	BR 6	141-112	0.57	
MEAN		131.6-99.7	3.90		128.9-94.5	2.00	49%
STD. ERROR		2.7 4.1	0.85		2.9 4.3	0.29	

TABLE 5

CHEMOTEST RELEASE BEFORE AND AFTER HYOSCINE METHYL BROMIDE

DOG NO.	BEFORE			AFTER			% INHIBITION OF C.A. RELEASE BY -HMB
	TEST	CHANGE OF PO_2 (mmHg)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)	TEST	CHANGE OF PO_2 (mmHg)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)	
7	CR 1	76-35	13.79	CR 3	185-40	0.49	
	CR 2	220-8	18.98	CR 4	185-31	-0.59	100%
10	CR 1	160-26.5	1.72	CR 3	202-10	1.29	
	CR 2	202-25.8	10.67	CR 4	175-39	8.74	16%
				CR 5	205-25.5	5.65	
12	CR 1	215-51	0.25*	CR 4	208-4	1.39	
	CR 2	202-35.5	4.44	CR 5	195-23	0.77	
	CR 3	180-38.5	4.22	CR 6	238-44	1.67	
				CR 7	228-28	2.88	61%
16	CR 1	115-46	2.09*	CR 5	114-22	1.36	
	CR 2	101-35	-0.71*	CR 6	106-27	0.79	
	CR 3	125-26	2.31				
	CR 4	120-21	3.42				62%
MEAN		160.6-27.0	7.44		185.5-26.7	2.22	
STD. ERROR		17.6 3.5	2.23		12.6 3.7	0.81	70%

TABLE 6

BAROTEST RELEASE BEFORE AND AFTER HEXAMETHONIUM AND HYOSCINE METHYL BROMIDE

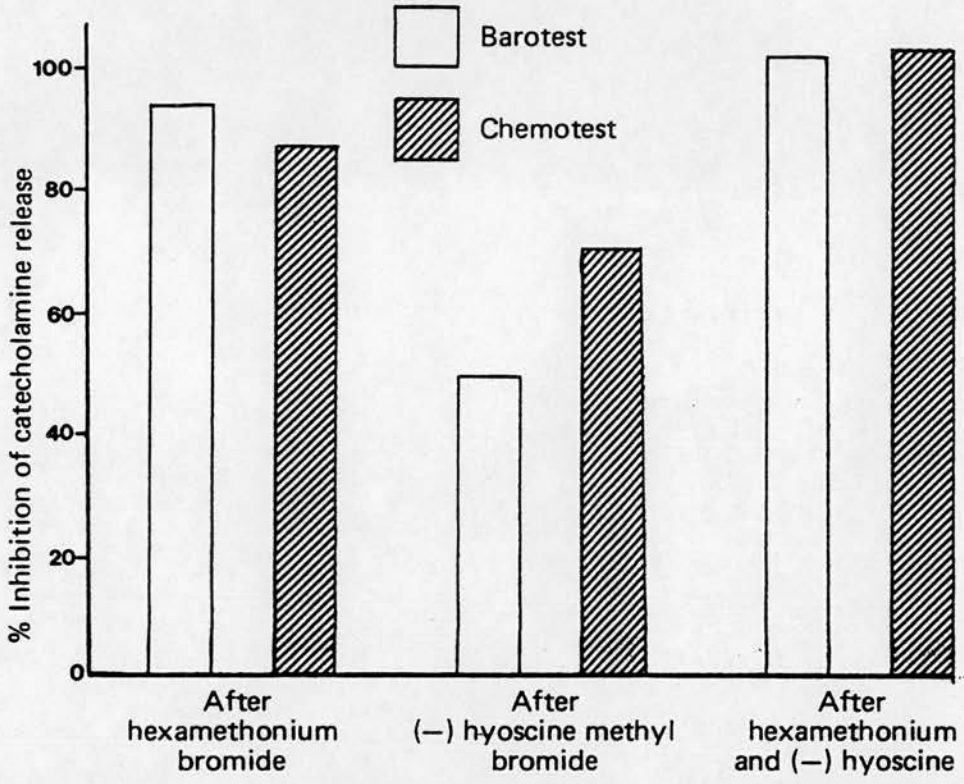
DOG NO.	BEFORE			AFTER			% INHIBITION OF C.A. RELEASE BY C_6 + -HMB
	TEST	CHANGE OF CPP (mmHg)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)	TEST	CHANGE OF CPP (mmHg)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)	
6	BR 1	132-99	9.12	BR 5	125-95	-0.03	
	BR 2	129-99	16.32				100%
16	BR 1	140-102	1.48	BR 7	140-101	-0.05	
	BR 2	140-111	1.82				
	BR 3	140-123	3.10				102%
MEAN		136.2-106.8	6.37		132.5-98.0	-0.04	
STD. ERROR		2.4 4.6	2.84		7.5 3.0	0.01	101%

TABLE 7

CHEMOTEST RELEASE BEFORE AND AFTER HEXAMETHONIUM AND HYOSCINE METHYL BROMIDE

DOG NO.	BEFORE			AFTER			% INHIBITION OF C.A. RELEASE BY C_6 + -HMB
	TEST	CHANGE OF PO_2 (mm Hg)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)	TEST	CHANGE OF PO_2 (mm Hg)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)	
6	CR 1	205-41	16.67	CR 5	144-38	-0.74	
	CR 2	168-37	37.12				103%
16	CR 1	115-46	2.09*	CR 7	131-29	0.00	
	CR 2	101-35	-0.71*				
	CR 3	125-26	2.31				
	CR 4	120-21	3.42				100%
MEAN		154.5-31.3	14.88		137.5-33.5	-0.37	
STD. ERROR		20.0 4.7	8.10		6.5 4.5	0.37	102%

Figure 12 Inhibition of reflex catecholamine release by nicotinic and muscarinic antagonists.



The columns represent the percentage inhibition of the mean catecholamine release from the left adrenal gland in dogs, during baroreceptor and chemoreceptor tests, by hexamethonium bromide (10 mg/kg), (−) hyoscine methyl bromide (10 mg/kg) and both antagonists together, in groups of 7, 4 and 2 dogs respectively.

Figure 12 shows the adrenal response to both baroreceptor and chemoreceptor reflexes is dependent on both nicotinic and muscarinic transmission. Nicotinic transmission is the more dominant of the two.

The results also suggest a possible difference in the transmission of the baroreceptor and chemoreceptor reflexes. The baroreceptor reflex being more dependent on nicotinic transmission and the chemoreceptor reflex more dependent on muscarinic transmission. This is discussed.

The ratio of noradrenaline:adrenaline remained constant, about 1:4, in all samples assayed.

d) <u>The effect of hexamethonium bromide on sympathetic ganglionic transmission of vasomotor reflexes from the carotid baroreceptors and chemoreceptors of the dog</u> –

In one dog, no. 34, no adreno-lumbar vein large enough to cannulate was present. We were therefore unable to collect and assay adrenal venous blood.

We took the opportunity to repeat an experiment of the type described by Henderson and Ungar (1978) which measures vascular resistance changes in a perfused hind limb following stimulation of the carotid baroreceptor and chemoreceptor reflexes. The only difference between our technique and that described by Henderson

and Ungar (1978) is the anaesthetic used. - The results are discussed with this point in mind.

Baroreceptor and chemoreceptor tests were matched to give similar rises in hind limb perfusion pressure. Changes in hind limb perfusion pressure were then measured during baroreceptor and chemoreceptor tests before and after hexamethonium bromide (10mg/kg), guanethidine (2mg/kg) and phentolamine (10mg/kg); added in that order. The hexamethonium was still effective when the phentolamine was added.

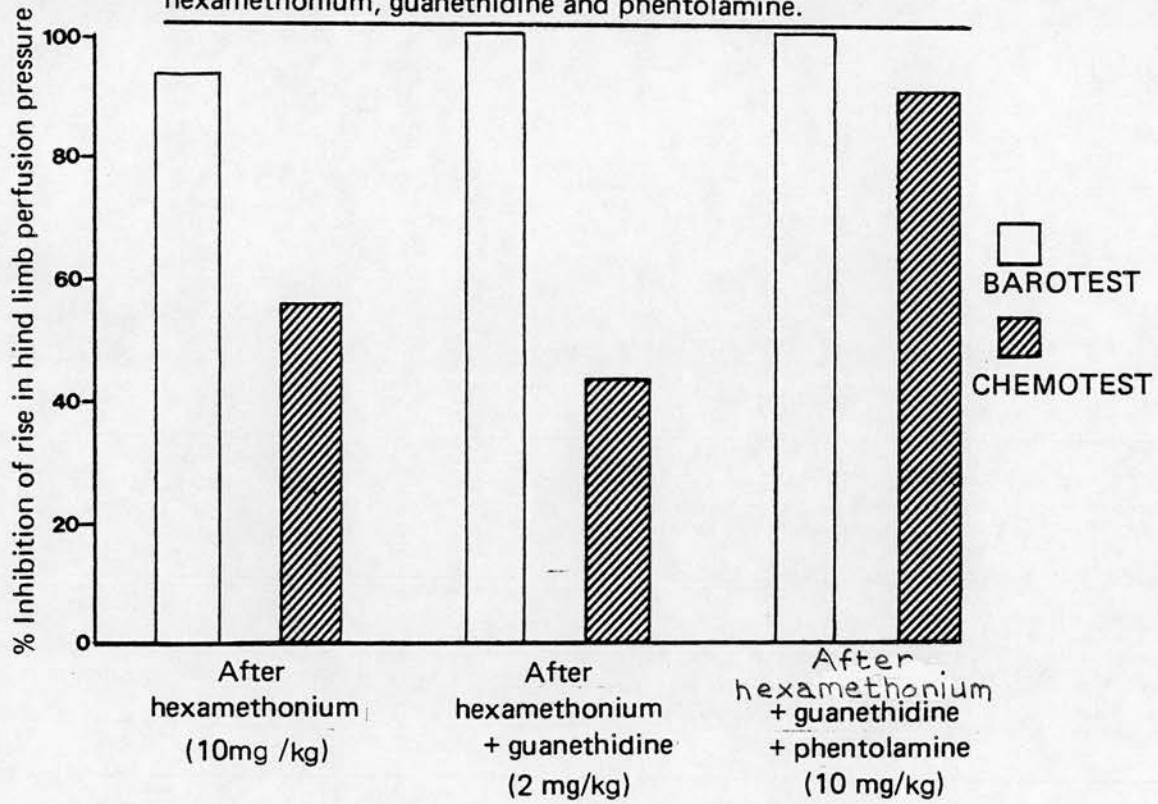
The results, expressed as percentage inhibition of the rise in hind limb perfusion pressure, are presented in figure 13.

Hexamethonium bromide almost completely inhibited the rise in hind limb perfusion pressure during baroreceptor tests but only inhibited the chemoreceptor response by about 50-60%.

Addition of guanethidine produced total inhibition of any residual response to the baroreceptor test but did not alter the chemoreceptor response remaining after blockade by hexamethonium.

Subsequent addition of phentolamine completely abolished any rise in hind limb perfusion pressure to either baroreceptor or chemoreceptor tests.

Figure 13 Inhibition of reflex increase in hind limb perfusion pressure by hexamethonium, guanethidine and phentolamine.



The results are taken from Dog no. 34.
The columns represent the percentage inhibition of the increase in hind limb perfusion pressure, which occurs during baroreceptor and chemoreceptor tests, by hexamethonium bromide; either alone or in combination with guanethidine and phentolamine.

Discussion

Feldberg, Minz and Tsudzimura (1934), found the response of the cat adrenal medulla to acetylcholine and splanchnic nerve stimulation could be abolished by a combination of nicotine and atropine but not by either drug alone. This led to the discovery that drugs acting at muscarinic receptors in the cat selectively control adrenaline release whilst drugs acting at the nicotinic receptors in the cat selectively control noradrenaline release form the adrenal medulla (Douglas and Poisner (1965), Rubin and Miele (1968) and Critchley (1976)).

The possibility of separate physiological roles for the two types of receptors in the cat was supported by Anichkov et al (1960) and Critchley, Ellis and Ungar (1980) who showed that carotid baroreceptor and chemoreceptor activity selectively regulates adrenal release of noradrenaline and adrenaline respectively. Critchley et al (1980) also demonstrated total inhibition of the baroreceptor response by a dose of hexamethonium bromide which did not alter the chemoreceptor response. This finding is also supported by the early work of Tenney (1956), who found that hexamethonium did not inhibit the adrenal catecholamine release nor the increase in systemic blood pressure in response to breathing 12-33% carbon dioxide.

This suggests the existence of two separate pathways in the cat, one nicotinic and one muscarinic, mediating the baroreceptor and chemoreceptor reflexes, namely,

Baroreceptor reflex ——> Nicotinic ——> Noradrenaline release

and, Chemoreceptor reflex ——> Muscarinic ——> Adrenaline release

In the dog baroreceptor and chemoreceptor activity regulate the total output of catecholamines but not the ratio of noradrenaline:adrenaline. However both muscarinic and nicotinic receptors, similar to the cat, are present in the canine adrenal medulla and both regulate catecholamine release under normal physiological conditions (Kayaalp and Türker (1968), (1969), Kayaalp and McIsaac (1969)). To investigate the possibility of two distinct pathways, one nicotinic and one muscarinic, mediating baroreceptor and chemoreceptor reflexes to the canine adrenal medulla, we studied the reflex release of catecholamines in the presence of nicotinic and muscarinic antagonists.

In the dog we found that both baroreceptor and chemoreceptor reflexes are more sensitive to nicotinic blockade and less sensitive to muscarinic blockade. However, muscarinic blockade still produces a marked reduction in the catecholamine release in response to both baroreceptor and chemoreceptor reflex stimulation. The situation in the dog is therefore not as simple as it is in the cat where a clear division between baroreceptor and chemoreceptor reflexes is found. For the dog we must postulate that both nicotinic and muscarinic receptors are involved in the transmission of the baroreceptor and chemoreceptor reflex to the adrenal medulla. None-the-less, the results do indicate some slight selectivity of the baroreceptor and chemoreceptor reflexes for nicotinic and muscarinic receptors respectively.

Other work also suggests reflex/receptor selectivity in the dog. In the one experiment (Dog 34) where we measured changes in hind limb perfussion pressure, hexamethonium selectively inhibited the baroreceptor response compared to the chemoreceptor response, even after administration of guanethidine. This implies that transmission of the baroreceptor reflex by the sympathetic ganglia and also by the adrenal medulla is predominently nicotinic. Henderson and Ungar (1978), who conducted a full study with this type of experiment using both hexamethonium bromide and hyoscine methyl bromide, demonstrated that sympathetic ganglion synaptic transmission during the baroreceptor reflex is mediated by nicotinic receptor activation and transmission evoked by chemoreceptor stimulation involves predominently muscarinic receptor activation. Henderson and Ungar (1978) report that guanethidine completely abolishes any change in hind limb perfusion pressure in response to either the baroreceptor or chemoreceptor reflex; indicating no involvement of adrenal catecholamines in the hind limb response. This is odd, as intravenous administration of adrenaline and noradrenaline at doses equivalent to that released from the adrenal medulla during reflex stimulation would undoubtedly result in vasoconstriction. A possible explanation of why we still see a chemoreceptor response in the hind limb following hexamethonium and guanethidine, is that the chemoreceptor reflex has a large muscarinic component, which releases catecholamines from the adrenal medulla of dogs under chloralose-urethane anaesthesia, but may even inhibit catecholamine release from dogs under pentobarbitone anaesthesia, as used by Henderson and Ungar (1978). (Effects of pentobarbitone anaesthesia on

the chemoreceptor reflex are reported in this thesis). Whilst this would explain Henderson and Ungar's lack of response to chemoreceptor stimulation following guanethidine it would not explain the lack of response to the baroreceptor reflex. Henderson and Ungar have not published figures for guanethidine effects and state that close arterial injection of guanethidine to the hind limb abolishes the response to chemoreceptor stimulation. The baroreceptor response is not mentioned. It may therefore be possible that some residual response to stimulation of the baroreceptor reflex did remain following guanethidine but was at the time considered to be insignificant. Alternatively in the animals used, the baroreceptor reflex may not have been strong enough to evoke catecholamine release sufficient to effect the hind limb response.

Further evidence in support of baroreceptor and chemoreceptor reflexes being mediated by nicotinic and muscarinic receptors respectively comes from the work of Shepherd's group (Pelletier (1972), Pelletier and Shepherd (1972), Webb-Peploe and Shepherd (1968)) and Professor James Black (personnal communication from Critchley (1976)). Professor Black has demonstrated that nicotinic agonists selectively constrict arteries and muscarinic agonists selectively constrict veins in the cat. Shepherd's group have shown the baroreceptor reflex to selectively constrict arteries and the chemoreceptor reflex to selectively constrict veins in the dog hind limb, i.e.

Baroreceptor reflex ——> nicotine ——> arteries.

Chemoreceptor reflex ——> muscarinic ——> veins.

If two separate pathways common to the cat and the dog do exist, one mediated by nicotinic receptors and the other by muscarinic receptors, then how is each pathway selectively triggered?

The simplest explanation of two separate pathways to the chromaffin cells of the adrenal medulla and to the sympathetic ganglion is two separate nerves or nerve fibres, one nicotinic and one muscarinic. Although this is possible there is no anatomical or electrophysiological evidence to support it. The most likely possibility is for different firing patterns within one nerve to relay different reflexes and stimulate different receptors.

The work of Volle's group, (Takeshige and Volle (1962), (1963), Takeshige et al (1963), DeGroat and Volle (1963), Volle (1963)) on the response of the sympathetic ganglia of the cat to acetylcholine, has clearly demonstrated an early excitatory postsynaptic potential (EPSP), which is susceptible to blockade by the standard nicotinic ganglionic blockers, and a late EPSP which is resistant to nicotinic block but is blocked by small doses of atropine. The late, or slow, EPSP is similar to that produced by muscarine. (Ambache et al (1956), Sanghvi and Unna (1963), Jones and Trendelenburg (1965)). Volle (1963) also noted that prior conditioning of the ganglia with tetanic stimulation enhanced the firing produced by muscarine and muscarinic agonists; whereas without prior conditioning large doses of acetylcholine were necessary to activate the late atropine sensitive asynchronous discharge in postganglionic fibres.

From this we can form a hypothesis to explain separate activation of nicotinic and muscarinic receptors by one neural route. The slow EPSP's of the muscarinic type would be expected to summate with rapid non-synchronous firing of the chemoreceptor type, the ganglia or adrenal medulla being at the same time conditioned by the continuous firing to produce slow EPSP's, whereas the short, fast, nicotinic EPSP would summate with synchronous firing of the baroreceptor type. The reflex induced nerve impulses would thereby dictate the receptor to be activated.

It is unfortunate that most of the work on catecholamine release in response to different patterns of nervous impulses has been done in the calf (Edwards et al (1980), Edwards (1981)a,b) as, although the bovine adrenal medulla possesses both nicotinic and muscarinic receptors, (Kayaalp and Neff (1979)) only stimulation of the nicotinic receptors is reported to release catecholamines. (Wilson and Kirshner (1977)). I found only one paper claiming muscarinic stimulation of catecholamine release, (Hawthorne and Mohd. Adnan (1981)), which claimed release by carbachol in the presence of 2×10^{-6}M tubocurarine. However, in another paper reporting a similar experiment, tubocurarine at 4×10^{-5}M was reported to completely abolish any catecholamine release stimulated by carbachol. (Fisher et al (1981)). None-the-less, Edwards has shown selective release of adrenaline and noradrenaline is possible by simply altering the pattern of nerve stimulation. I would agree therefore with his final statement in the discussion from Edwards (1981) b) which reads,

"This finding obviates the necessity to involve separate efferent pathways to the separate types of chromaffin cell (Palkama (1962, 1964)), although the existence of separate pathways remains an open question."

Stimulation of the muscarinic receptor in the bovine adrenal medulla does not release catecholamines but has been reported to inhibit the release of catecholamines (Derome et al (1981), Lemaire et al (1980)) and has been suggested to act by increasing cyclic GMP levels. (Lemaire et al (1981)). However, whilst it is generally accepted that cyclic GMP is increased in the presence of muscarinic agonists it is not widely accepted that the increase in cyclic GMP and stimulation of the muscarinic receptor are synonymous. (Schneider et al (1979), Yanagihara et al (1979), El-Fakahany and Richelson (1981)).

Kebabian et al (1975) whilst studying the bovine sympathetic ganglion, postulated that an increase in the neural concentration of cyclic GMP in sympathetic ganglia is involved in the mechanism which generates slow EPSP's in the postganglionic neurons. Support for this theory comes from work on the frog, (Weight et al (1974), Nishi and Koketsu (1968)) whilst evidence against it comes from work on the rat, (Frey and McIsaac (1981)) and the rabbit. (Dun et al (1977), (1978)). In conclusion there appear to be both quantitative and qualitative differences among species in the mechanisms which generate cyclic GMP responses.

Whether cyclic GMP is responsible for mediating muscarinic responses in either the dog or cat sympathetic ganglia or adrenal medulla is not

known. However, a single neural pathway may exist which mediates both nicotinic and muscarinic responses and cyclic GMP may be involved in the muscarinic transmission. In the bovine adrenal medulla the same could be true where muscarinic stimulation, without releasing catecholamines per se, may modify the release or even alter the ratio of noradrenaline:adrenaline released.

C. The Role of the Pituitary-Adrenocortical Axis in Reflex Responses of the Adrenal Medulla of the dog

Results

The release of adrenaline and noradrenaline from the adrenal medulla in response to long duration chemoreceptor tests was studied in 4 groups of dogs.

The first group consisted of 5 control dogs; the second of 5 dogs with denervated left adrenal glands; the third of 1 dog pretreated with cycloheximide (50mg/kg), and the fourth group of one dog also pretreated with cycloheximide (50mg/kg) but in addition the left adrenal gland was denervated.

In all dogs the catecholamine release from the left adrenal gland was measured before and during baroreceptor tests and at timed intervals before, during and after long chemoreceptor tests. The long chemoreceptor tests were of 20 minutes duration in the first and second groups and of 10 minutes duration in the third and fourth groups and consisted of lowering the PO_2 of the carotid perfusate from above 100mmHg to about 45mmHg in all dogs.

The abolition of the adrenal response to baroreceptor tests, while the pressor response remained, was taken as evidence of complete denervation of the left adrenal glands.

The results of all 4 groups of dogs are presented in figure 14.

Figure 14 Responses of dogs to baroreceptor and long chemoreceptor tests.

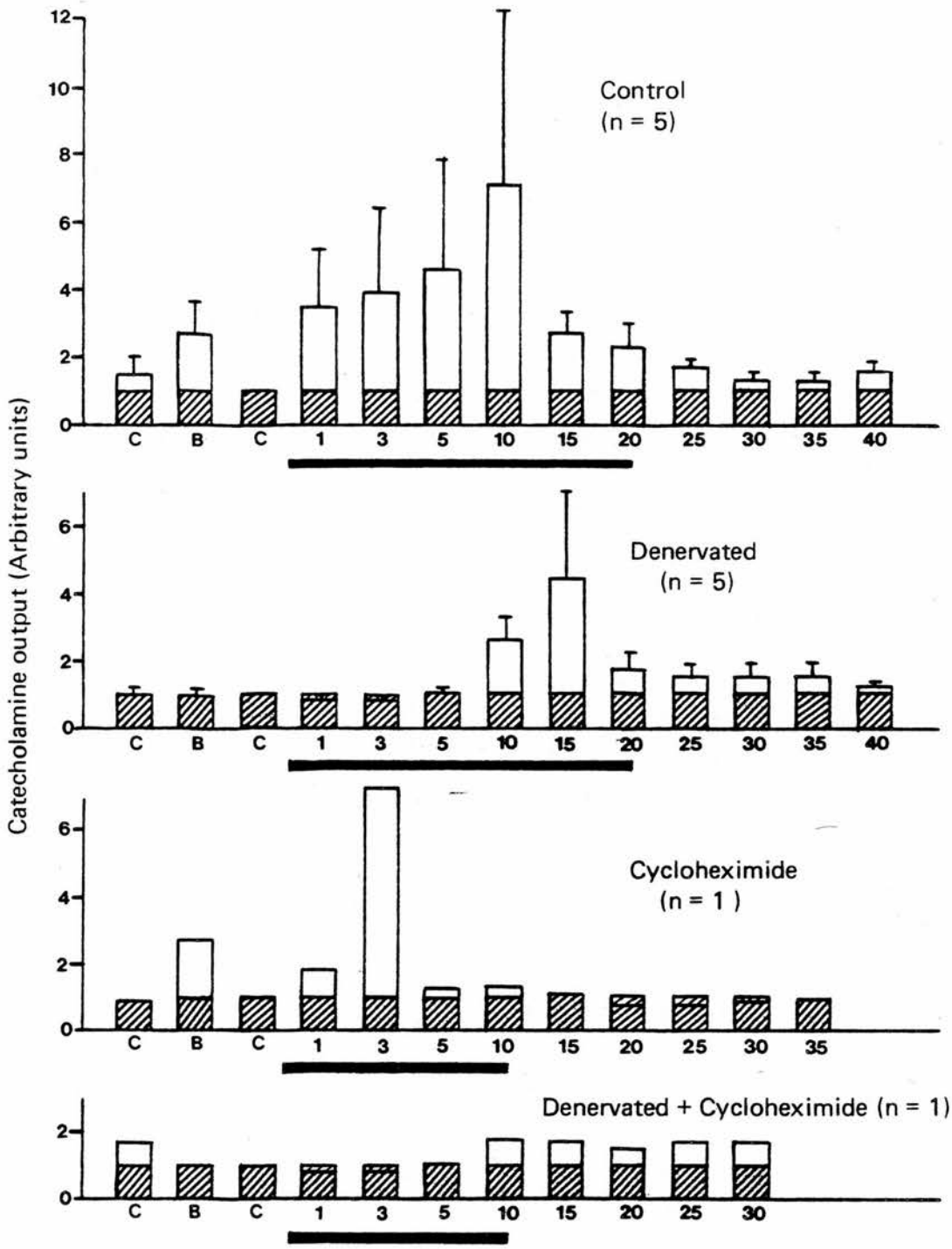


Figure 14. Responses of dogs to baroreceptor and long chemoreceptor tests. The columns represent the mean output of catecholamines from the left adrenal gland during control periods (C), baroreceptor tests (B), and at set times from the start of the long chemoreceptor test (shown in minutes). Catecholamine output preceeding the long chemoreceptor test was given the value of 1. The vertical bars represent the S.E. of the mean. The solid horizontal bars represent the duration of the long chemoreceptor tests in which the PO_2 of the carotid perfusate is reduced from above 100 mmHg to about 45 mmHg. The panels, from top to bottom, show the mean responses of, 1 untreated dogs, 2 dogs with denervated left adrenal glands, 3 dogs given cycloheximide (50 mg/kg) and 4 dogs with denervated left adrenal glands also given cycloheximide (50 mg/kg). The hatching represents the control catecholamine output immediately preceeding the chemoreceptor test.

Adrenal venous collections were not always taken at the times indicated in figure 14. When this was the case an estimate of the catecholamine release was made. This I did by assuming a linear relationship between catecholamine release and time between any two adjacent adrenal venous collectons. The release was thus estimated from adrenal venous collections made either side of the time period indicated.

Chemoreceptor Tests in control dogs (Group 1)

Dog numbers 22, 23, 26, 32 and 33.

In all 5 dogs baroreceptor tests produced marked increases in catecholamine output.

Long chemoreceptor tests, of 20 minute duration, produced a rapid rise in catecholamine output which was sustained throughout the stimulus and remained slightly elevated beyond 20 minutes after the stimulus.

Chemoreceptor Tests in dogs with denervated left adrenal glands (Group 2)

Dog numbers 27, 28, 29, 30 and 31.

Long chemoreceptor tests, of 20 minute duration, produced increases in catecholamine output which outlasted the stimulus. However, in contrast to dogs with innervated adrenal glands, no release was observed during the first 5 minutes of chemoreceptor stimulation or during baroreceptor tests.

The Effect of Cycloheximide on the response of dogs with innervated adrenal glands to chemoreceptor tests (Group 3)

Dog number 32.

Cycloheximide, a drug which inhibits the release of corticosteroids in response to corticotrophin (Garren, Ney and Davis (1965)), did not affect the immediate release of catecholamines occurring during chemoreceptor stimulation or baroreceptor tests, but abolished any catecholamine release occurring beyond 5 minutes of chemoreceptor stimulation.

The combined effect of Cycloheximide and Denervation on the adrenal response to chemoreceptor tests (Group 4)

Dog number 31.

Cycloheximide and denervation in combination completely abolished any increase in catecholamine output during baroreceptor tests and during the first 5 minutes of chemoreceptor stimulation. The increase in catecholamine output occurring beyond 5 minutes of chemoreceptor stimulation was greatly reduced but not abolished. This implies the cycloheximide block in this dog was incomplete.

The effect of exogenous corticotrophin (Synacthen) on adrenomedullary catecholamine output

Dog number 18, 19 and 21-26.

In 6 dogs Synacthen was infused intravenously either at 25μg/min for 10 minutes, 25μg/min for 1 minute or 6.25μg/min for 4 minutes.

At all infusion rates Synacthen produced a large increase in catecholamine output which was maintained for up to and even beyond 60 minutes. The time to peak catecholamine output varied between dogs, being almost immediate in some and up to 50 minutes after infusion in others.

(-)Hyoscine methyl bromide (10mg/kg) was administered to one dog (Dog no. 24) between infusions of Synacthen (25μg/min for 1 minute). No reduction in catecholamine release to Synacthen infusion was observed.

In two dogs (Dogs no. 25, 26) Synacthen (25μg/min for 1 minute) was infused following administration of hexamethonium bromide (10mg/kg) and (-)hyoscine methyl bromide (10mg/kg). Hexamethonium and hyoscine reduced the resting release of catecholamines to zero and Synacthen infusion produced no increase up to 20 minutes following infusion. Samples were not taken for long enough to say conclusively that all response to Synacthen was abolished. However, in all other dogs Synacthen infusion did produce an increase in catecholamine output by 20 minutes.

Hydrocortisone Assay

We attempted to assay adrenal venous hydrocortisone levels within this series of experiments. However, our attempts appeared to be unsuccessful as we could demonstrate no real change in hydrocortisone levels even during Synacthen infusion, which is used clinically to test adrenal cortical function. Our methods are presented in Appendix 1 and the results are included in Appendix 2, but no attempt has been made to interpret them.

Discussion

In our earlier experiments we investigated the response of the adrenal medulla to short hypoxic stimuli, localised to the carotid bifurcations, lasting 90 seconds. We always found the release of catecholamines began and ended sharply with the beginning and end of the stimulus (allowing time for hypoxic blood to wash out of the perfusion system) as one would expect of a reflex response mediated by the autonomic nervous system. Critchley and Ungar (1974) observed however, that if the stimulus was prolonged to 20 minutes the release of catecholamines outlasted the stimulus by at least a further 30 minutes, although both the adrenal glands and the central nervous system remained perfused with well oxygenated blood throughout the experiment. A secretory response which outlasts the stimulus by more than a few minutes is more likely to be mediated by a humoral mechanism than a neural mechanism. The crucial test of such a hypothesis is to divide the motor nerves supplying the effector organ.

We successfully achieved this in 5 dogs and were indeed unable to abolish the release of catecholamines in response to chemoreceptor stimulus although the initial, presumably neurally mediated, release was abolished. It is possible that this response, like tachycardia and vasodilation, could be a secondary neural or chemical consequence of increased pulmonary ventilation evoked by carotid body hypoxia (Daly and Scott (1963)). However, this possibility is excluded in our expeirments since the lungs were ventilated at a constant rate and the vagi were cut.

We took as a working hypothesis the involvement of the pituitary-adrenocortical axis since we know that carotid chemoreceptor stimulation, dependent on intact nerves, releases corticotrophin from the anterior pituitary and thus corticosteroids from the adrenal cortex of the dog (Anichkov et al (1960), Lau and Marotta (1969), (1970), Marotta (1972)) and also the rat, (Marks et al (1965)). To test this hypothesis we performed two types of experiment. Firstly we investigated the action of cycloheximide, a drug known to inhibit the release of corticosteroids in response to corticotrophin, (Garren et al (1965)), on the release of catecholamines evoked by carotid body hypoxia. The immediate release of catecholamines during both baroreceptor and chemoreceptor tests was not impaired by cycloheximide. However, the sustained release in response to chemoreceptor tests was abolished by cycloheximide, indicating the involvement of corticosteroids in mediating the late component of catecholamine release in response to carotid body hypoxia. Secondly we investigated the adrenomedullary response to infusion of exogenous corticotrophin, as Synacthen - a synthetic analogue of corticotrophin. Synacthen produced large increases in catecholamine output. This was also found by Critchley and Ungar (1974) who in addition demonstrated inhibition of the corticotrophin response by cycloheximide. Combining my results from dogs with denervated adrenal glands and Critchley's (1976) results from infusing Synacthen we can show a similar time course for the adrenomedullary response to Synacthen infusion (25μg over 20 minutes) and to a 20 minute chemoreceptor test in dogs with denervated adrenal glands. My own data using Synacthen is not compatible as I rapidly infused large doses. However, if my results are compared from the end of the infusion where 25μg of Synacthen was given in both cases, then the results are similar.

Finally, Critchley et al (1975) have shown that hydrocortisone releases catecholamines from the isolated perfused canine adrenal gland, this release not being blocked by cycloheximide (Henderson (1980)).

In summary, the evidence supporting involvement of the pituitary-adrenocortical axis in reflex responses of the canine adrenal medulla is as follows:-

1. The release of catecholamines from the adrenal medulla, in response to carotid body hypoxia, may outlast the stimulus.

2. Denervation of the adrenal gland abolishes the immediate release of catecholamines in response to carotid body hypoxia but the prolonged release remains.

3. The prolonged release of catecholamines is abolished by cycloheximide.

4. Both corticotrophin 'in vivo' and hydrocortisone in the isolated perfused adrenal gland release adrenomedullary catecholamines.

5. Carotid body hypoxia has been shown to release corticotrophin from the anterior pituitary.

Few other workers have investigated the effect of adrenocortical function on the release of catecholamines, although many have investigated its action on the synthesis of adrenaline. I was only able to find reports from three groups which I considered relevant to this discussion.

Roffi et al (1966) demonstrated that corticotrophin and hydrocortisone increased adrenaline release from the adrenal glands of hypophysectomised rats, indicating possible involvement of the pituitary-adrenocortical axis in adrenomedullary catecholamine release in the rat.

The only piece of relevant work to come from the dog is by Wurtman et al (1968). Wurtman et al (1968) studied the release of adrenomedullary catecholamines in response to insulin-induced hypoglycaemia and interpreted their results solely with regard to changes in the synthesis of adrenaline induced by corticosteroids. Their results, which are expressed as percentage of adrenaline in adrenal venous plasma can be recalculated to show both the resting output of adrenaline and noradrenaline and also the release stimulated during insulin hypoglycaemia. This reveals that corticotrophin in the hypophysectomised dog increases the output of both adrenaline and noradrenaline at rest and particularly during insulin hypoglycaemia. This would support our findings whilst the potentiation of the release during hypoglycaemia would suggest increased synthesis of adrenaline and also noradrenaline by corticosteroids or alternatively that induction of PNMT is secondary to release of adrenaline.

Evidence against involvement of the pituitary-adrenocortical axis in the release of catecholamines from the adrenal medulla comes from work on the conscious calf by Edward's group (Edwards et al (1975), Bloom et al (1977a,b)). They have shown marked increases in hydrocortisone release from the adrenal gland during mild hypoxia or hypercapnia without any or only minimal catecholamine release respectively. This shows that

hydrocortisone per se exerts no significant effect on catecholamine release in the conscious calf. Henderson (1980) investigated the effects of infusing hydrocortisone to isolated feline adrenal glands and reports no change in catecholamine output.

It is therefore possible that species differences exist in the adrenomedullary response to corticosteroids.

In conclusion, there are two components to the response of the canine adrenal medulla to carotid body hypoxia:- 1. The rapid component dependent on an intact nerve supply to the adrenal gland but independent of adrenocortical function, and 2. The delayed component requiring an intact pituitary-adrenocortical axis but independent of the motor nerves to the gland.

Also, in this series of experiments the degree of hypoxia induced was less than that during short 'neural' chemoreceptor tests. Although I have not investigated the threshold levels of hypoxia required to release catecholamines either by neural or humoral mechanisms it is possible that the humoral mechansim may have the lower hypoxic threshold and could thus have the greater physiological significance.

D. Effects of Indomethacin on Blood Pressure, Catecholamine Release and Adrenal Blood Flow in the anaesthetised, laparotomised dog

A problem we encountered in studying adrenomedullary reflexes was low and poorly maintained blood pressures. The blood pressure fell following laparotomy and handling of the viscera, necessary for cannulation of the adrenal gland. Terragno et al (1977) reported laparotomy to cause release of prostaglandins and that indomethacin, given after laparotomy restores the blood pressure. In view of this finding we gave indomethacin (5mg/kg), in divided doses to dogs before and after laparotomy.

Results

Indomethacin treatment resulted in a mean blood pressure of about 100mmHg, the dogs requiring only small volumes of dextran to maintain it for up to 3 hours. Without indomethacin a mean blood pressure of less than 70mmHg resulted and large volumes of dextran were required to maintain it. Indomethacin was therefore administered to several dogs to maintain blood pressure.

Feuerstein et al (1979) reported that indomethacin increased the adrenal output of catecholamines from the cat adrenal medulla in response to haemorrhage. We therefore investigated the effect of indomethacin on catecholamine release at rest and in response to baroreceptor tests.

We found catecholamine output at rest and the increased release during baroreceptor tests to be reduced by indomethacin.

	C.A. output at rest	Incremental output during baroreceptor tests.
Control	23.91 ± 5.37	11.91 ± 2.44
Indomethacin	4.31 ± 0.55	3.35 ± 0.68

(Catecholamines are expressed as ng $kg^{-1}min^{-1}$)

This is opposite to the findings of Feuerstein et al (1979) and is discussed.

In addition to the effects of indomethacin on blood pressure and catecholamine release, we observed a reduction of adrenal blood flow at rest and also a reduction of the increase which occurs following chemoreceptor and baroreceptor tests. - This surprised us as the increase in systemic blood pressure in response to baroreceptor and chemoreceptor tests was if anything larger after indomethacin. We would have anticipated a larger increase in adrenal blood flow to occur with the larger rise in blood pressure.

To investigate this observation fully we constructed resting pressure-flow curves for control and indomethacin treated dogs (see figure 15). The data used to construct the curves, or more accurately regression lines, is contained in tables 8 and 9.

Figure 15 shows the linear regression lines, with 95% confidence limits, of adrenal blood flow on systemic blood pressure.

TABLE 8

Resting Blood Pressure, Adrenal Blood Flow and Catecholamine Release in dogs not given indomethacin

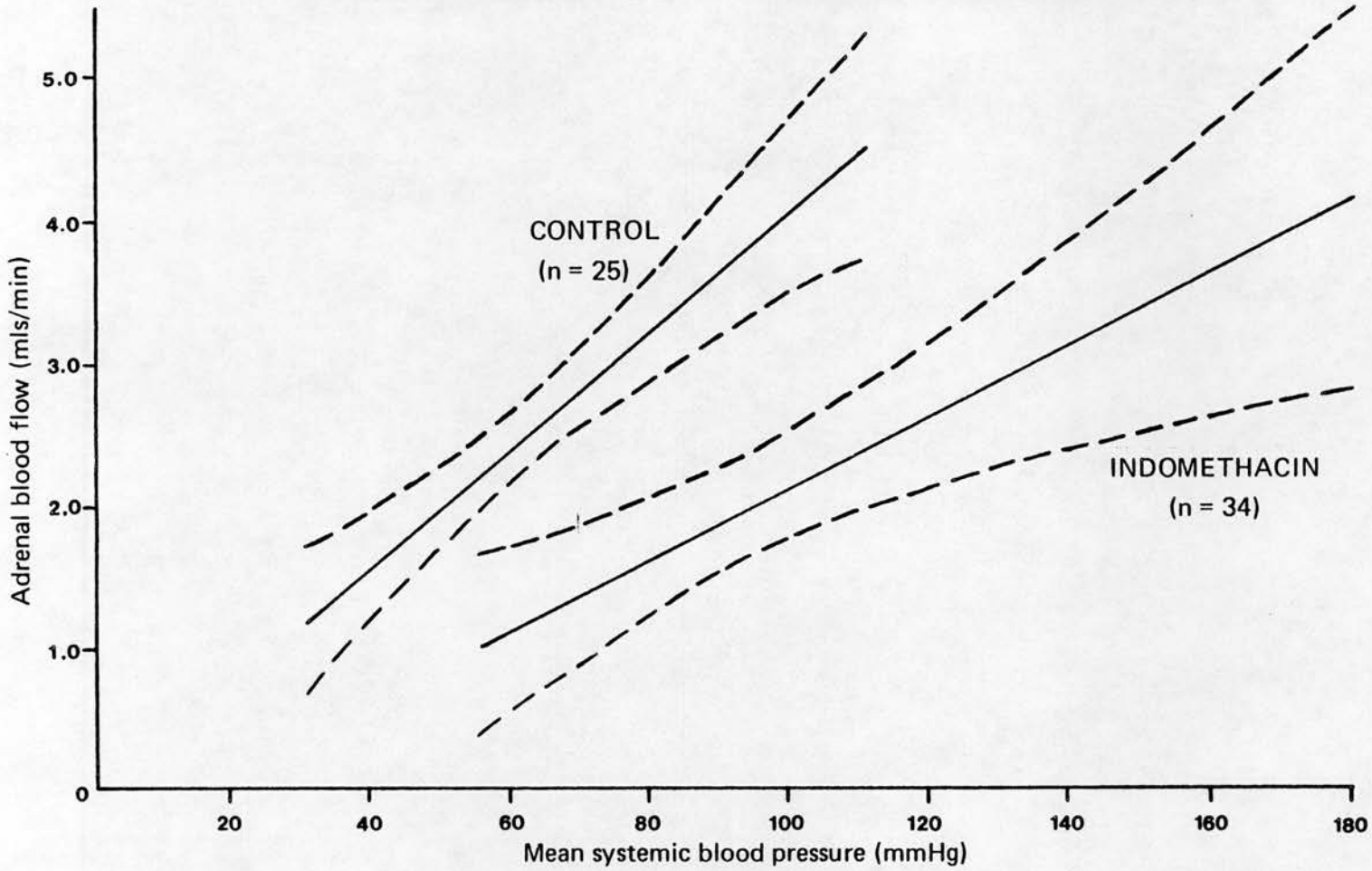
DOG NO.	S.B.P. (mmHg)	ADRENAL BLOOD FLOW (mls/min)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)
6	60	2.25	8.52
	49	1.40	12.04
	64	1.60	19.64
	57	1.55	31.08
7	42	1.10	7.71
	42	1.25	3.05
	53	2.45	19.55
	44	1.40	9.68
8	49	3.25	3.07
	42	2.15	4.32
	38	1.30	3.73
	31	0.65	3.44
9	77	2.75	28.43
	61	2.25	24.13
	63	2.60	19.82
	65	3.15	20.83
19	68	3.45	7.68
	66	3.20	14.24
	64	3.50	24.16
20	55	2.93	21.60
	69	3.30	18.10
	66	2.50	18.00
22	108	3.75	85.05
	86	3.35	93.34
	78	3.60	96.58

TABLE 9

Resting Blood Pressure, Adrenal Blood Flow and Catecholamine Release in dogs given indomethacin

DOG NO.	S.B.P. (mmHg)	ADRENAL BLOOD FLOW (mls/min)	C.A. RELEASE ($ng.kg^{-1}min^{-1}$)
10	178	6.80	13.89
	122	3.95	4.52
	129	2.50	3.59
	102	1.70	1.62
11	70	0.85	3.23
	91	0.85	5.83
	83	0.50	9.10
	54	0.60	9.79
12	99	2.90	3.93
	90	2.50	5.15
	84	1.65	2.12
	75	1.15	2.25
	57	1.25	1.41
13	92	3.45	0.68
	69	2.60	0.49
	70	2.25	0.92
	85	2.65	2.52
14	79	2.50	10.45
	81	2.45	7.41
	105	2.55	4.98
	80	1.90	5.16
16	82	1.40	2.03
	60	0.90	2.91
	99	0.90	4.58
	136	1.10	6.69
	122	0.90	3.37
	126	0.85	4.55
	98	1.00	4.55
17	100	2.50	1.17
	74	1.65	1.50
	78	1.45	2.59
	87	1.65	1.74
	75	1.45	2.11
18	73	2.05	9.73

Figure 15 Resting pressure/flow curves for left adrenal gland, with and without indomethacin



Linear regression of adrenal blood flow on systemic blood pressure, with indomethacin (r = 0.54) and without (r = 0.77). All lines show 95% confidence limits.

Indomethacin produced a highly significant depression of the resting pressure-flow curve. This suggests prostaglandins are involved in maintaining adrenal blood flow.

The increases in adrenal blood flow observed during baroreceptor and chemoreceptor tests would presumably be largely due to increases in systemic blood pressure. To investigate the effect of indomethacin on the relationship between catecholamine release and adrenal blood flow we must first correct the changes in adrenal blood flow for any changes occurring in systemic blood pressure. This is done using the resting pressure-flow curves of figure 15.

The corrected flow changes are calculated thus:-

Change in flow = Actual flow change - (Pressure change x Gradient)
(corrected)

where gradient is the gradient of the relevant resting pressure-flow curve.

In order to obtain accurate corrected flow values we require an accurate measure of the change in systemic blood pressure. As the blood pressure response to chemoreceptor tests is unstable and irregular (see figure 10) we used data obtained from only baroreceptor tests; where the systemic blood pressure response is a regular 'square wave'.

The regression lines with 95% confidence limits are presented in figure 16. The data used to construct the lines is contained in tables 10 and 11.

TABLE 10

Changes in Blood Pressure, Adrenal Blood Flow and Catecholamine Release during Barotests in dogs not given indomethacin

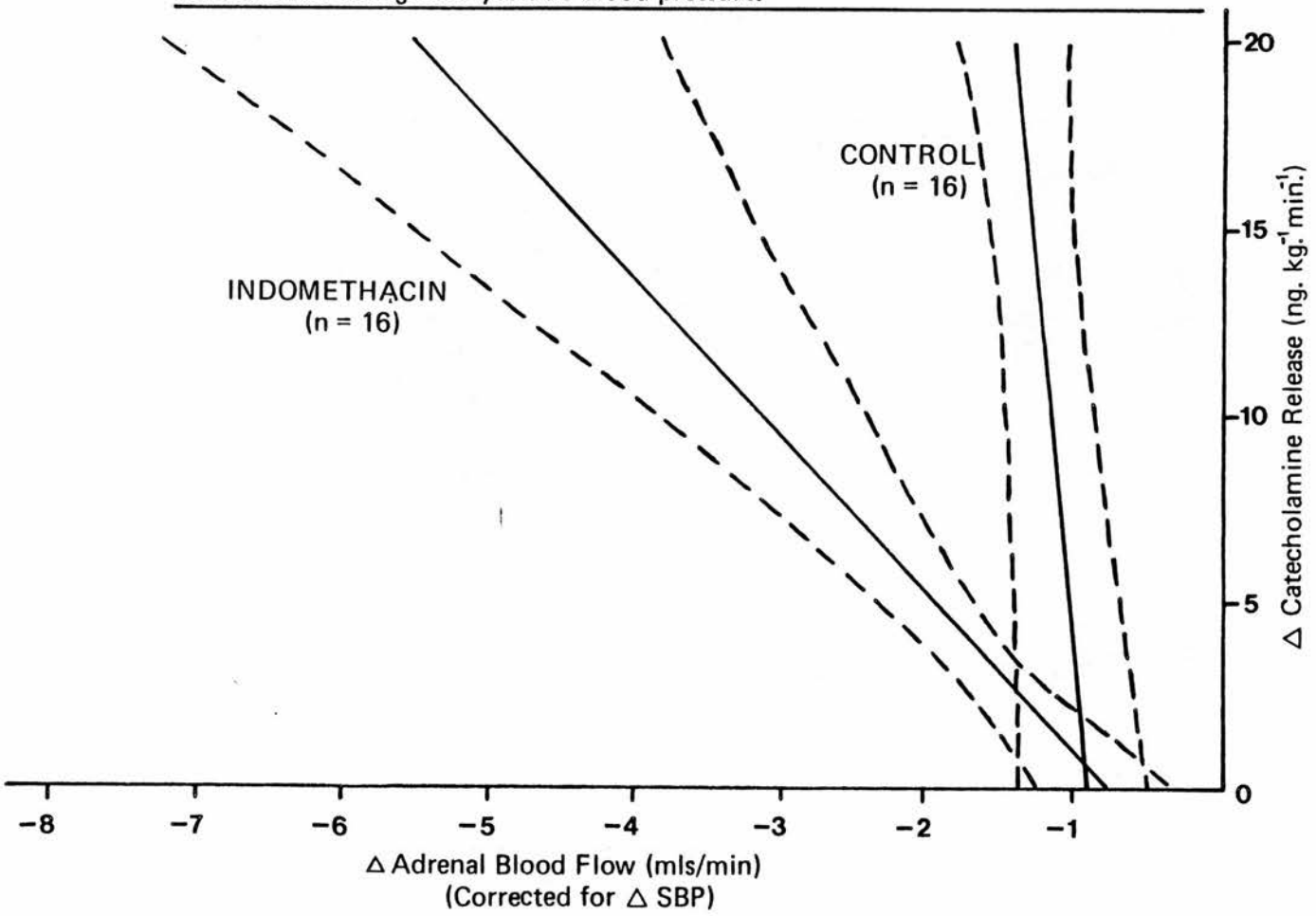
DOG NO.	TEST	Δ FLOW (ml/min)	Δ SBP (mmHg)	Δ FLOW - (ΔSBP x 4.22 x 10^{-2}) (ml/min)	Δ C.A. ($ng.kg^{-1}.min^{-1}$)
6	BR 1	0.50	49	-1.57	9.12
	BR 2	0.75	56	-1.61	16.32
7	BR 2	0.05	8	-0.29	1.89
	BR 3	-0.25	16	-0.93	2.55
8	BR 1	0.75	41	-0.98	1.77
	BR 2	1.20	44	-0.66	9.37
9	BR 1	1.00	38	-0.60	7.02
	BR 2	1.25	42	-0.52	13.79
19	BR 1	1.30	87	-2.37	6.71
	BR 2	0.95	66	-1.84	15.76
	BR 3	1.50	59	-0.99	4.89
20	BR 1	1.11	47	-0.87	11.60
	BR 2	0.66	47	-1.32	9.60
	BR 3	1.22	58	-1.23	13.20
22	BR 1	0.75	56	-1.61	38.30
	BR 2	0.70	64	-2.00	28.61

TABLE 11

Changes in Blood Pressure, Adrenal Blood Flow and Catecholamine Release during Barotests in dogs given indomethacin

DOG NO.	TEST	Δ FLOW (ml/min)	Δ SBP (mmHg)	Δ FLOW - (ΔSBP x 2.53 x 10^{-2}) (ml/min)	Δ C.A. ($ng.kg^{-1}min^{-1}$)
10	BR 1	-1.05	122	-4.14	9.72
	BR 2	0.75	106	-1.93	5.72
11	BR 1	0.30	83	-1.80	6.22
	BR 2	-0.05	61	-1.59	0.90
12	BR 1	0.25	75	-1.65	5.61
	BR 2	0.60	76	-1.32	4.44
13	BR 1	0.95	126	-2.24	3.04
	BR 2	1.50	147	-2.22	6.16
14	BR 1	0.40	51	-0.89	0.17
	BR 2	0.30	57	-1.14	1.88
16	BR 1	0.10	44	-1.01	1.48
	BR 2	0.30	77	-1.65	1.82
	BR 3	0.30	45	-0.84	3.10
17	BR 1	0.15	51	-1.14	0.88
	BR 2	0.65	67	-1.05	2.29
18	BR 2	1.50	104	-1.13	0.13

Figure 16 Plot of barotest stimulated catecholamine release against change in adrenal blood flow, corrected for changes in systemic blood pressure.



Linear regression of change in adrenal blood flow (corrected for pressure) on stimulated catecholamine release, with indomethacin (r = 0.80) and without (r = 0.46). All lines show 95% confidence limits.

The results from figure 16 after indomethacin imply a marked vasoconstriction associated with the release of catecholamines which is not seen without indomethacin. This suggests prostaglandins are normally released with catecholamines and maintain adrenal blood flow by opposing their constricting action.

Statistical analysis using scatter plots reveals a heavy weighting of one point used to construct the indomethacin regression line. (-4.14, 9.72 - from table 11). If this point is not used for the analysis then a similar picture to figure 16 results but the separation of the two regression lines is much less and significance can only just be claimed at the 90% level. However, I have no reason to exclude this point. It is unfortunate therefore that no other baroreceptor tests released similar amounts of catecholamines to strengthen the analysis.

Effect of Synacthen infusion on blood pressure, catecholamine release and adrenal blood flow

Three dogs (nos. 22-24) received a 25µg infusion of Synacthen.

In all dogs an increase in catecholamine release and adrenal blood flow occurred without any increase in systemic blood pressure. This implies ACTH directly increases adrenal blood flow. The increase could be mediated by prostaglandins released with the catecholamines but this is unlikely to completely explain it, as baroreceptor tests are unable to show any increase in adrenal blood flow when pressure changes are corrected for.

Synacthen infusion was investigated in two dogs treated with indomethacin (nos. 18 and 19). However, the adrenal glands of these dogs were rendered partly inactive and unreliable by attempted denervation using lignocaine. (see 'Methods - Denervation of left adrenal gland').

The Effect of Indomethacin on the release of PGI_2 from the adrenal medulla of the anaesthetised dog

The results are presented in full in Appendix 2, Dog no. 20.

In one dog, no. 20, prostaglandin 6-oxo-$F_{1\alpha}$, the stable metabolite of PGI_2, was assayed, in addition to catecholamines, in adrenal venous blood. PGI_2 was also assayed in one arterial sample taken at the start of the experiment.

The level of PGI_2 in the arterial sample was 0.11ng/ml.

As this is less than the concentration of PGI_2 found in adrenal venous samples, PGI_2 is released from the adrenal gland.

Increases in PGI_2 levels occur with increases in catecholamine release during baroreceptor tests.

Indomethacin (5mg/kg) abolishes the increase in PGI_2 occurring during baroreceptor tests but does not alter the increase in catecholamine output.

We attempted to assay PGE_2 also, but the concentration of PGE_2 was too high, even after indomethacin, and we did not have enough sample left to repeat the assay with a suitable dilution.

PGI_2 was assayed by 'radio-immunoassay'.

The Effects of Indomethacin and PGE_2 on catecholamine release from isolated canine adrenal glands

A. The Effect of Indomethacin on catecholamine release stimulated by nicotine

Indomethacin (1µg/ml) had no effect on the resting release of catecholamines.

The adrenal response to infusions of nicotine (10^{-5}M - 10^{-3}M) was variable. However, in two glands indomethacin appeared to reduce the catecholamine release in response to nicotine whereas in one gland the release appeared to be potentiated.

B. The Effect of PGE_2 on catecholamine release

A PGE_2 solvent blank produced no release of catecholamines. PGE_2, 5-600ng/ml produced marked catecholamine release each time it was infused. Although the response of the glands was again variable evidence of a dose related effect was found (Gland no. 7).

C. The Effect of Hexamethonium on catecholamine release stimulated by PGE_2

In this series of experiments PGE_2 consistently increased catecholamine output and was not blocked by hexamethonium.

Discussion

From the results in figure 15 it is clear that prostaglandins do play a role in maintaining adrenal blood flow in the dog, under normal conditions. If the results from figure 16 are accepted, although statistical evidence for any difference after indomethacin is weak, then it would appear that prostaglandins are released with increasing concentrations of catecholamines and could have a role counteracting the vasoconstriction associated with catecholamine release. In addition prostaglandins may be inhibiting platelet aggregation, which is known to occur in the presence of high concentrations of catecholamines such as occurs in the adrenal vein. The results from dog 20 support this hypothesis, as prostaglandin I_2 (PGI_2) release is found to increase with catecholamine release. Catecholamines are also reported to release prostaglandins from the kidney of the rabbit (Needleman et al (1974)) and the dog (Terashima et al (1974)). The release of PG's from the rabbit kidney is inhibited by α-receptor blockade and this may be involved in the mechanism of PG release from the adrenal gland. However, PG release from the canine kidney is not α blocked.

Rather than considering the effect of catecholamines on PG release most authors have investigated the effects of PG's on catecholamine release, as this is obviously much easier to do in the adrenal gland. My

findings in the dog with indomethacin, a prostaglandin synthetase inhibitor (Ferreira et al (1971)), indicate catecholamines would be released by PG's. I have also supported this 'in vitro', showing marked catecholamine release from the isolated perfused canine adrenal gland in response to infusions of PGE_2.

Evidence has been produced of specific binding sites for PGE_1 and PGE_2 in the bovine, ovine and human adrenal medulla (Karaplis and Powell (1981), Dazord et al (1974)) but I am unable to find any evidence of a binding site in the canine adrenal medulla. However, in addition to my own work Kayaalp and Türker (1967) have shown PGE_1 to release catecholamines from the adrenal medulla of the dog. The question of whether PG's, particularly PG's of the E series, release or inhibit catecholamine output from the adrenal medulla is still under debate. On one hand Gutman and Boonyaviroj claim PGE_2 inhibits, and indomethacin potentiates, catecholamine release in human, (Boonyaviroj and Gutman (1977)), rat (1975), bovine (1979) and feline (Feuerstein, Feuerstein and Gutman (1979)) adrenal medulla. The mechanism of action of PGE_2 is claimed by them to be inhibition of adenyl cyclase (Gutman and Boonyaviroj (1979)). Their findings agree with the hypothesis of Hedqvist, who found PG's of the E series antagonised sympathetic responses due to partial inhibition of noradrenaline release (Hedqvist (1970), Hedqvist and Brundin (1969)) whereas PG-synthetase inhibitors facilitate adrenergic responses and enhance noradrenaline release (Hedqvist et al (1971), Samuelsson and Wennmalm (1971)). Hedqvist concluded that PG's of the E series may limit noradrenaline release from sympathetic nerves by a presynaptic inhibitory mechanism. However, much of the data supporting this hypothesis is based on 'in vitro' studies in which isolated artificially perfused organs and artificial stimulation

techniques are used. Also, in most studies, the release of tritiated noradrenaline and not endogenous noradrenaline was measured. Whereas Hedqvist's theory may be true for some sympathetic nerves it is not substantiated in the sympathetic ganglia of the cat (Kayaalp and McIsaac (1968)). The ganglion is a site of cholinergic transmission similar to the adrenal medulla and not adrenergic transmission as at sympathetic nerve endings. I feel therefore that Hedqvist's work cannot really be compared with the release of catecholamines from the adrenal medulla.

Whereas Gutman and Boonyaviroj claim PG's inhibit and indomethacin potentiates catecholamine release from the adrenal medulla, other workers have found the reverse to be true; in the rat (Feuerstein et al (1981), (1982)), cat (1981) and human (Newman and Brodows (1982)). Feuerstein, who has worked with Gutman, has published papers supporting both argu ments from the one species, the cat. However, Feuerstein now appears to be convinced that PG's do release catecholamines under a variety of conditions and has criticised the earlier negative findings as lacking any in vivo support.

Whilst the question remains unclear in some species, I have found no reports suggesting PG's inhibit catecholamine release from the adrenal gland of the dog. I therefore suggest that, in the dog, PG's of the E series release catecholamines from the adrenal medulla whilst the PG-synthetase inhibitor, indomethacin, inhibits release.

It is generally accepted that PGE_2 and PGI_2 cause vasodilation of blood vessels. However, little work has been done to directly measure changes in adrenal blood flow in response to PG's. What evidence there is supports my finding that PG's produce an increase in adrenal blood flow.

Phernetton and Rankin (1979) have shown PGI_2 reduces adrenal vascular resistance in maternal and foetal sheep. In addition to this Houck and Lutherer (1981) have demonstrated indomethacin reduces adrenal vascular resistance and blood flow in dogs. However, they found the adrenal gland was able to maintain its blood flow during haemorrhagic hypotension to 50mmHg, even in the presence of indomethacin. They tested regulation of adrenal blood flow to haemorrhage under a variety of conditions and concluded that the maintenance of adrenal blood flow during haemorrhagic hypotension was independent of the autonomic nervous system, prostaglandin synthesis, histamine recpetors and an intact pituitary-adrenal axis. However, all these systems did influence adrenal blood flow. - A possibility not investigated by Houck and Lutherer was regulation by G.I.P., gastric inhibitory polypeptide. There is some evidence to suggest that G.I.P., a compound similar in structure to V.I.P., vasoactive intestinal polypeptide, has a significant effect on adrenal blood flow in the dog at physiological concentrations (Andersen et al (1982)).

The remaining evidence suggesting involvement of PG's in increasing adrenal blood flow is indirect and comes largely from the work of Varga et al, using ACTH and indomethacin. They found ACTH produces increases in both adrenal and ovarian blood flow in the dog which is blocked by indomethacin. (Varga et al (1976, 1978, 1979), Stark and Varga (1975), Horváth et al (1981)). My results support their finding that ACTH increases adrenal blood flow but unfortunately I was unable to test the response in the presence of indomethacin owing to damaged preparations (see 'Results').

Stark et al (1965, 1968) found the ACTH induced increase in adrenal blood flow continued even after corticosteroid increase had ceased. However, cycloheximide was found to block both the increase in adrenal blood flow and steroidogenesis in response to ACTH. The two responses were not considered to be related as steroidogenesis ceased some 2 hours before cycloheximide had any effect on adrenal blood flow.

PGE_2 has also been shown to stimulate corticosteroidogenesis (Louis et al, (1976), Flack et al (1969), Saruta and Kaplan (1972)) and it has been suggested that PGE_2 is involved in mediating ACTH induced steroidogenesis as well as increases in adrenal blood flow. As PGE_2 appears to induce steroidogenesis and increase corticosteroids, it is possible that part of the adrenomedullary response to PGE_2 is mediated by corticosteroids; as corticosteroids have been shown to increase catecholamine release from the canine adrenal medulla (Critchley et al (1975)). Whilst corticosteroids may mediate part of the PGE_2 induced catecholamine release they certainly do not mediate it all, as I have found PGE_2 retrogradely infused to the canine adrenal gland, thus avoiding any cortical influence, still produces marked increases in catecholamine release. Nonetheless, an action of PGE_2 on catecholamine release mediated by corticosteroids 'in vivo' cannot be excluded.

Summary of Results

1. Hexamethonium bromide produces small but consistent increases in the percentage of noradrenaline released at rest.

2. No other evidence of selective release was found. The ratio of noradrenaline:adrenaline remained constant at 1:3.5.

3. Hexamethonium bromide significantly inhibits the adrenal response to both baroreceptor and chemoreceptor tests. The inhibition is consistently greater during baroreceptor tests.

4. (-)Hyoscine methyl bromide significantly inhibits the adrenal response to both baroreceptor and chemoreceptor tests but to a lesser extent than hexamethonium. The mean inhibition of the chemoreceptor test was the greater.

5. Hexamethonium bromide and hyoscine methyl bromide in combination abolish any adrenal response to baroreceptor and chemoreceptor tests.

6. Both nicotinic and muscarinic receptors are involved in the transmission of the baroreceptor and chemoreceptor reflexes to the adrenal medulla. Some selectivity of the baroreceptor and chemoreceptor reflexes for nicotinic and muscarinic receptors respectively is shown.

7. The release of catecholamines from the adrenal medulla in response to carotid body hypoxia may outlast the stimulus.

8. After denervation of the adrenal gland the immediate release of catecholamines in response to carotid body hypoxia is abolished, but the prolonged release remains.

9. The prolonged release of catecholamines is abolished by cycloheximide.

10. Denervated glands in combination with cycloheximide do not release catecholamines in response to carotid body hypoxia.

11. Corticotrophin markedly increases catecholamine release from the adrenal medulla.

12. Indomethacin maintains systemic blood pressure following laparotomy.

13. Indomethacin inhibits catecholamine release both at rest and during baroreceptor tests.

14. Prostaglandin E_2 releases catecholamines from the isolated perfused canine adrenal gland.

15. Prostaglandin I_2 is released from the adrenal gland of the dog during baroreceptor tests and at rest. Indomethacin abolishes release.

16. Indomethacin depresses the resting pressure-flow curve of the adrenal gland and inhibits the rise in adrenal blood flow which occurs during baroreceptor and chemoreceptor tests.

17. Corticotrophin (Synacthen) increases adrenal blood flow without any increase in systemic blood pressure.

Conclusions

1. The canine adrenal medulla does not selectively release adrenaline or noradrenaline in response to chemoreceptor or baroreceptor reflex stimulation, as found in the cat. If selective release of catecholamines occurs at all in the dog, the changes in the noradrenaline:adrenaline ratio are so small that they are difficult to detect.

2. Transmission of the baroreceptor and chemoreceptor reflex to the adrenal medulla of the dog consists predominently of nicotinic receptor activation with a large subsidiary muscarinic component. There is some evidence to suggest selectivity of the baroreceptor reflex for nicotinic receptors and the chemoreceptor reflex for muscarinic receptors. - This is not conclusive.

3. There are two components to the response of the adrenal medulla to arterial chemoreceptor stimulation.

   a) A rapid component requiring an intact nerve supply to the adrenal gland but independent of adrenocortical function, and

   b) A delayed component requiring an intact pituitary - adrenocortical axis but independent of the motor nerves to the gland.

4. Prostaglandins play a role in maintaining adrenal blood flow in the dog. - I would also suggest prostaglandins are released with catecholamines and maintain adrenal blood flow by opposing the constrictor action of the catecholamines.

5. Prostaglandin E_2 releases catecholamines from the canine adrenal medulla. Whether this will prove to have any physiological significance remains to be determined.

Appendix 1

The Assay of Hydrocortisone in Adrenal Venous Plasma

Introduction:-

Previous workers in this laboratory have implicated hydrocortisone in the release of catecholamines from the canine adrenal gland, especially in situations of prolonged stimulation of the arterial chemoreceptors. (Critchley and Ungar (1974), Critchley, Henderson, Moffat, Ungar, Waite and West (1975)). As I continued the investigation into the humoral control of catecholamine release from the adrenal medulla I decided it would be useful to assay adrenal venous blood, not only for catecholamines, but also for hydrocortisone.

Selection of Technique

Three different methods for the assay of plasma hydrocortisone are currently in use:- Colorimetric, fluorimetric and competitive protein binding radioassay. The colorimetric or Silber-Porter procedure (see Peterson, Karrer and Guerra (1957)) does not correlate well with results from other techniques. (Mejer and Blanchard (1973)). The colorimetric method measures 17-hydroxy-corticosteroids and the higher values this technique yields for plasma hydrocortisone may be caused by other substances in plasma which do not affect fluorimetric methods, e.g. ketones.

The competative protein binding assay is highly specific for hydrocortisone and is undoubtedly the method of choice. (Murphy (1967), Nugent and Mayes (1966), Few and Cashmore (1971), Malinowska, Hardy and Nathanielsz (1972)). However, our laboratory was not equipped

to make use of this type of assay. Therefore, as second choice, we selected the fluorimetric assay of hydrocortisone. Fluorimetry is a well established technique in our laboratory as it is used routinely for the assay of adrenaline and noradrenaline. Therefore, little effort was entailed in setting up the fluorimetric procedure for hydrocortisone.

The fluorimetric assay of hydrocortisone

The quantitative determination of adrenal 11-hydroxy-corticosteroids from the fluorescence induced by ethanolic sulphuric acid was first demonstrated by Sweat (1954). Since then many modifications have been introduced, resulting in publication of a variety of different methods, e.g. Silber, Busch and Oslapas (1958), Guillemin et al (1958), Mattingly (1962), DeMoor et al (1970), Kitabchi and Kitchell (1970), Meyer and Blanchard (1973)). For easier reading Rubin (1970) has summarised most of the modifications.

We selected the method of Meyer and Blanchard (1973); as their assay offered the simplest procedure, with sensitivity, accuracy and specificity equal to that of any of the other methods. Meyer and Blanchard's procedure is based on the method of Kitabchi and Kitchell (1970), which they have simplified by eliminating centrifugation, processing samples consecutively and by prealkalizing in standards, blanks and plasma standards.

Experimental Protocol for Hydrocortisone Assay

a) Adrenal venous sample

Adrenal venous blood is collected and the plasma separated and stored (see 'Experimental Protocol for Catecholamine Assay').

b) Extraction procedure

The plasma is thawed and the fibrin resuspended and mixed to ensure even distribution.

0.5mls plasma is transferred to a 15ml stoppered tube. (The remainder of the sample is stored for subsequent catecholamine estimation).

Samples are processed at 1 minute intervals.

1. Add 50µl of 0.25M Sodium hydroxide to the plasma.
2. Whirly-mix for 10 seconds.
3. After 4.5 minutes add 10mls of dichloromethane.
4. Whirly-mix for 25 seconds.
5. Centrifuge at 3000r.p.m. for 2 minutes to ensure phase separation.
6. Transfer 6mls of the lower organic phase to a clean 15ml stoppered tube.

c) Production of fluorescence

1. Add 4mls of 'fluorescence reagent' (see notes on assay) to the 6mls of organic phase.
2. Whirly-mix for 10 seconds.
3. After phase separation is complete (about 10 minutes) discard the upper organic phase and transfer the lower alcoholic phase to an appropriate cuvette or tube which will fit the 'Aminco-Bowman Spectrophotofluorimeter'.
4. 45 minutes after the addition of the 'fluorescence reagent' the fluorescence is read in the spectrophotofluorimeter. The emission wavelength is fixed at 530mμ and the excitation wavelength scanned from 400mμ to scatter at about 530mμ. The peak fluorescence is recorded at excitation wavelength 470mμ.

d) Blanks

Reagent blanks are run by substituting 0.5ml of distilled, deionised water for plasma.

e) Standards

Stock solution:- Hydrocortisone (10μg/ml) is dissolved in absolute ethanol and stored at $4^{o}C$.

Working standards of 1μg/ml are made from the stock solution by diluting with distilled, deionised water.

0.5ml of the working standard is substituted for plasma and assayed accordingly.

Plasma standards are also run by adding stock solution to plasma and comparing with plasma without stock solution added.

Notes on the Hydrocortisone Assay

(1) Volume of plasma used

Mejer and Blanchard (1973) used 1ml of plasma for their assay of serum 11-hydroxycorticosteroids. As I am assaying adrenal venous blood and corticosteroid levels are higher only 0.5ml of plasma has been used.

(2) Tubes used in assay

Stoppered tubes are essential to prevent spillage during mixing as large volumes are used. This is particularly important when mixing the fluorescence reagent as it is highly acidic and burns when in contact with skin.

(3) Sodium hydroxide reagent

Prealkalinization eliminates non-cortisol fluorescence from the sample without affecting the 11-hydroxycorticosteroids (Nielsen and Asfeldt (1967), Mejer and Blanchard (1973)). The concentration of sodium hydroxide used is the same for the method of Mejer and Blanchard. Mejer and Blanchard (1973) demonstrated that further increase in the concentration of sodium hydroxide did not reduce fluorescence. Also, prealkalinization times of 0 to 15 minutes produced no significant variation in fluorescence.

(4) Centrifugation to phase separate

Kitabchi and Kitchell (1970) introduced phase separating filter paper as a time saving device to separate the aqueous plasma phase from the organic extraction phase. As we did not have phase separating filter paper available when setting up the assay, a short spin in a bench centrifuge and careful removal of 6mls of organic phase, through the aqueous phase, was tried and found adequate. In fact, the filter paper contributes to the reagent blank value. When removing the organic phase particular attention must be paid to ensure that the extract remains free from moisture; thus avoiding the possible problem of spurious hydrocortisone values as a result of moisture uptake during extraction. (DeLange and Whittlestone (1970)).

(5) Fluorescence reagent

The fluorescence reagent comprises 6.5 volumes of sulphuric acid to 3.5 volumes of absolute ethanol.

To combine these reagents they are first cooled to 4°C and the concentrated sulphuric acid added slowly to the absolute ethanol, immersed in a bath of alcohol and dry ice to prevent the temperature exceeding 25°C.

This is a hazardous procedure and great care must be taken when synthesising this reagent.

The fluorescence reagent is very stable (2 months at 37°C) but does discolour with time. The reagent was stored at 4°C and renewed every 2 months or as required.

(6) Reading of fluorescence

Mejer and Blanchard (1973) examined the development of fluorescence with time. They found fluorescence took about 30 minutes to develop and was stable up to 1 hour. We therefore read the fluorescence at 45 minutes, the mid-point of the plateau in the fluorescence/ time curve. I found there to be no change in fluorescence when the reading was taken 5 minutes either side of this point.

In agreement with Koch, Edwards and Chilcote (1973) I find the fluorescence maxima for excitation and emission to occur at 470 and 530mμ respectively. Koch et al (1973) also found the minimum interference from non-specific fluorophors to occur at these wavelengths. Therefore, 470/530mμ is used for reading fluorescence rather than 470/520mμ as used by Mejer and Blanchard (1973). An example of the typical fluorescence spectra of a sample is presented (figure 17).

(7) Linearity of assay

The linearity of the assay was checked by assaying known standards of hydrocortisone in the range 20ng to 2μg. Working standards and plasma standards were compared and no difference was found.

Figure 17 Tracing of the fluorescence spectra of 11-hydroxycorticosteroids in an adrenal venous plasma sample. (Emission wavelength set to 530 mμ)

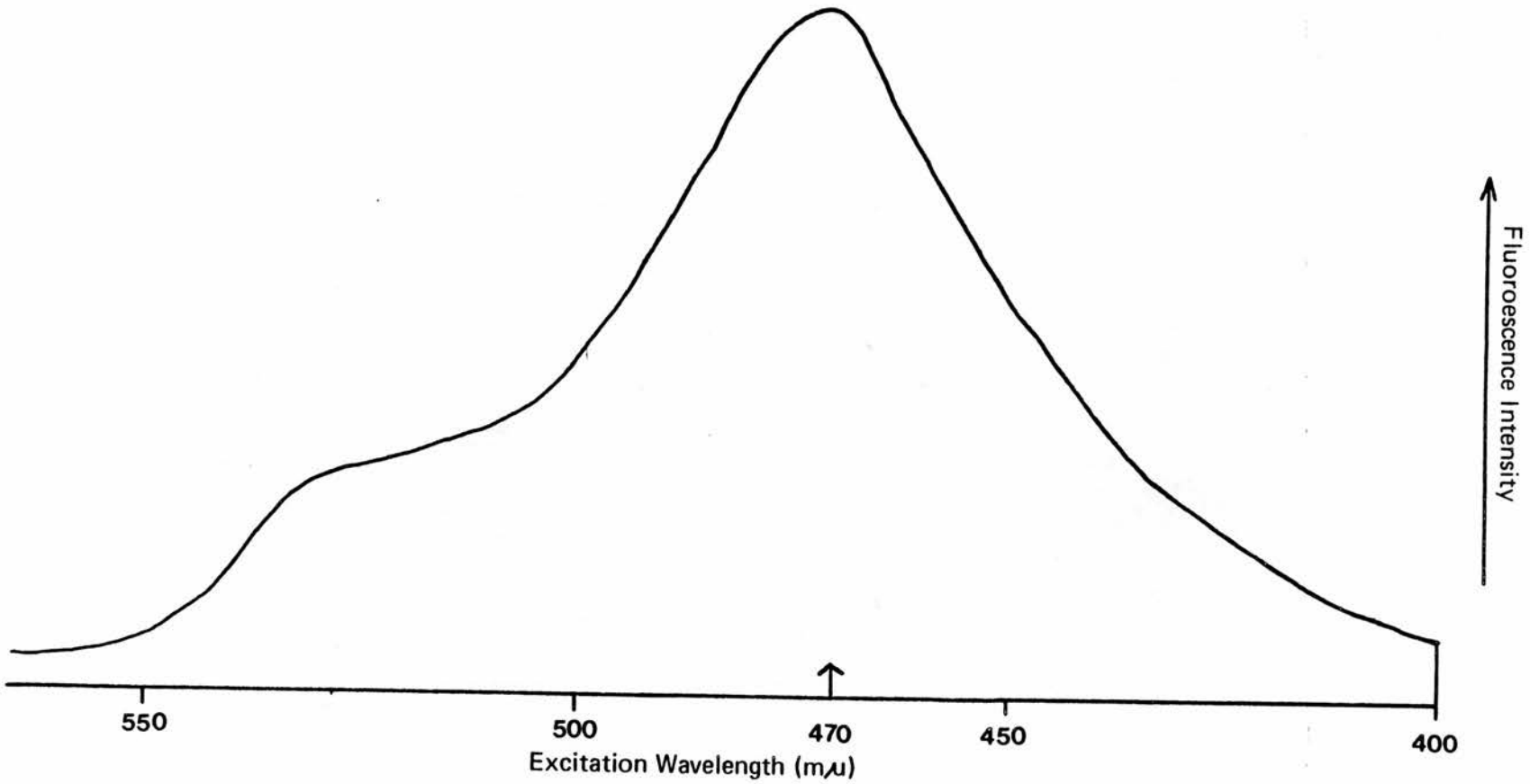
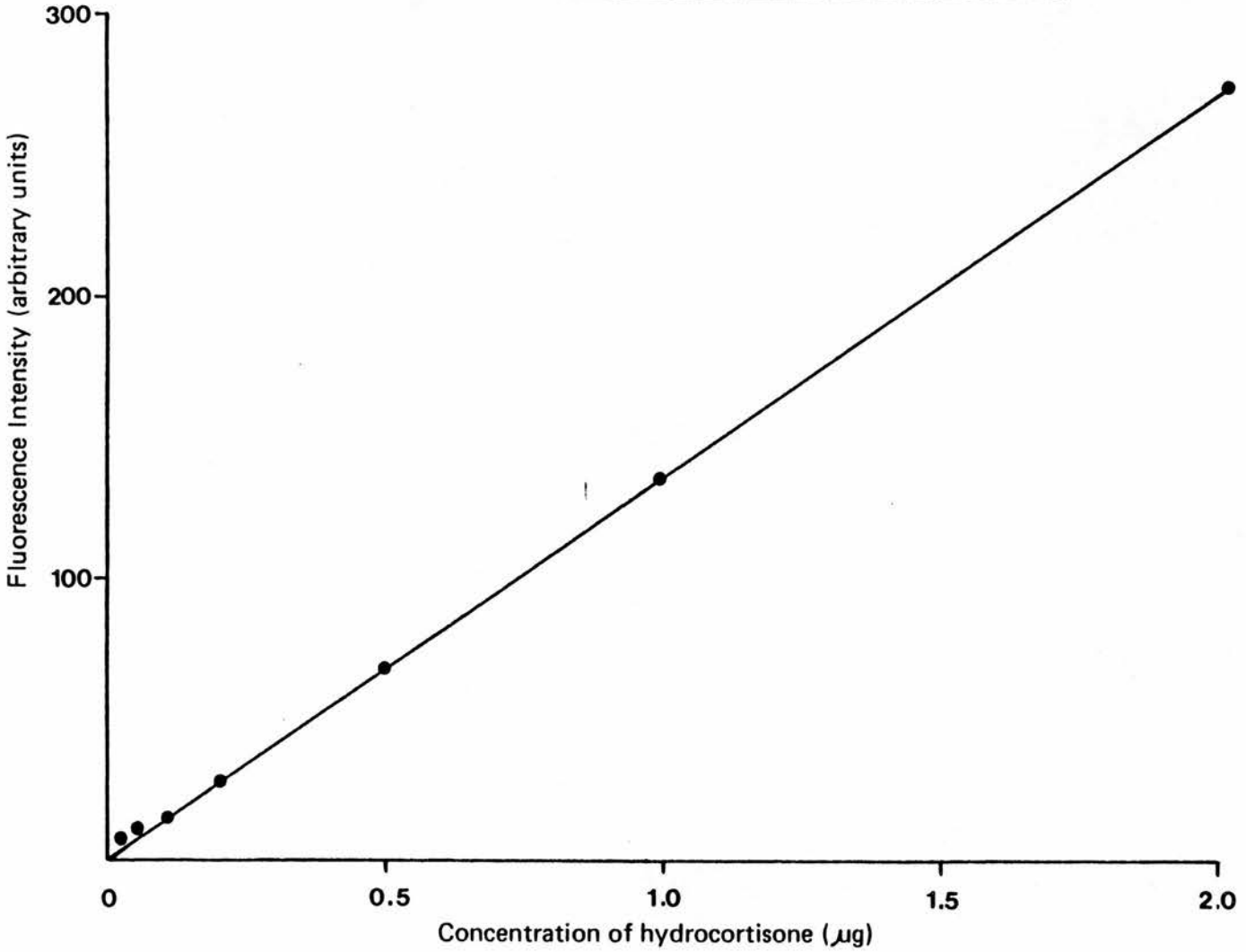


Figure 18 Calibration curve for hydrocortisone in plasma, fluorescence read at 470/530 mμ



The results obtained using plasma standards are presented in Figure 18.

I did not pursue the assay to its limit of sensitivity as the levels of corticosteroids in the samples assayed were high; however, Mejer and Blanchard (1973) claim that a standard of 5ng consistently gives fluorescence values higher than the reagent blank, when a spectrophotofluorimeter is used. (If a filter type fluorimeter is used 10ng is the limit of sensitivity).

(8) Specificity

Mejer and Blanchard (1973) have published a paper studying the specificity of their method. They identified four principal contaminants in the hydrocortisone containing extract of human plasma. These were corticosterone, triglyceride, cholesterol and non-specific fluorogens. Of the total fluorescence measured an average of 62% was due to hydrocortisone, 29.9% to corticosterone and 9.9% to the remainder. - This appears no less specific than any other fluorimetric technique available. In adrenal samples the level of hydrocortisone, and also corticosterone, is higher than in peripheral samples; therefore I would expect the other plasma fluorogens to contribute relatively less to the total fluorescence. Although hydrocortisone is the principal corticosteroid released from the adrenal gland corticosterone is also released in measurable quantities. The fluorescence spectra of these two

compounds are indistinguishable and the fluorescence intensity per microgram of corticosterone is about five times that of hydrocortisone. This implies the assay is only valid if the ratio of corticosterone to hydrocortisone remains constant. As I was aware of no evidence from the dog indicating selective release, the assay was used; but with reservations.

Results

11-Hydroxy-corticosteroids were measured in adrenal venous blood samples from twelve dogs. The results from each animal are reported in Appendix 2. 'Tables of Results from Whole Animal Experiments'.

Of the twelve dogs, from which samples were assayed for 11-hydroxy-corticosteroids, four were given an infusion of Synacthen (Ciba) with no other drug present; one receiving 250µg and the others 25µg. Synacthen, (tetracosactrin acetate), is a synthetic analogue of corticotrophin (ACTH), 1µg equivalent to about 1 I.U. of ACTH, possessing the complete therapeutic activity of cortioctrophin and indistinguishable from it in its action. Synacthen (25µg) would therefore be expected to produce a marked increase in adrenal venous hydrocortisone levels as has been shown with ACTH, e.g. Marotta, S.F. et al (1973). This however is not the case.

The resting levels of adrenal venous hydrocortisone lie within the range of published figures but, at best, only a modest and transient increase in hdyrocortisone occurs with Synacthen infusion.

As Synacthen is used clinically to test adrenal-cortical function and ACTH is known to release hydrocortisone from the canine adrenal cortex I must express doubt as to the reliability of the assay I have used.

A possible explanation of poor hydrocortisone release could lie in the differing fluorescence intensity produced by hydrocortisone and

corticosterone; (see 'Notes on the Hydrocortisone Assay - Specificity') such that a five unit increase in hydrocortisone accompanied by a one unit decrease in corticosterone would produce no change in total fluorescence, and thereby no change in measured corticosteroid. This explanation is doubtful as ACTH is reported to increase corticosterone levels as well as hydrocortisone; in fact, increased corticosterone has been used, in the rat, as an indicator of increased ACTH release, e.g. Stark, E. et al (1970). Also, Marotta and co-workers, a leading group in the study of adrenal corticosteroids, have used both the 17-hydroxy-corticosteroid colorimetric method (which does not measure corticosterone) and the 11-hydroxy-corticosteroid fluorimetric method and reported no obvious differences.

If the assay is valid then either the adrenal cortex of the dogs used were insensitive to Synacthen or were too depleted of corticosteroids, through surgical stress, to respond normally. The latter explanation may be true as resting corticosteroid levels, although within the published range for anaesthetised dogs, lie close to the lower limit of the range.

Owing to the dubious nature of the results obtained I have not used the corticosteroid figures to draw any conclusions and have presented the assay as an appendix rather than part of the main text.

Appendix 2

Tables of Results from Whole Animal Experiments

Key

Index

P = Sodium Pentobarbitone.

C/U = Chloralose/Urethane.

CYCLOHEX = Cycloheximide.

I = Indomethacin.

() indicates the indomethacin was given during the experiment rather than before.

HEX = Hexamethonium Bromide.

HYO = (-) Hyoscine Methyl Bromide.

() indicates which of HEX and HYO was administered second.

ACTH = Adrenocorticotrophic Hormone (Synacthen).

C.A.EST = Catecholamine estimation (Adrenaline and Noradrenaline)

HYD = Hydrocortisone (11-hydroxy-corticosteroids).

PG = Prostaglandin-6-OXO-$F_{1\alpha}$

Tables

No = refers to sample number

pO_2 and pCo_2 = Partial gas pressures in mmHg.

CPP = Carotid perfusion pressure.

SBP = Mean systemic blood pressure.

CA = Catecholamines (Adrenaline + Noradrenaline)

BR = Baroreceptor test.

CR = Chemoreceptor test.

CR′ = Sample collection immediately following CR.

LCR = Long chemoreceptor test.

INDEX OF PROCEDURES USED IN WHOLE ANIMAL EXPERIMENTS

DOG NO.	ANAESTHETIC		LONG CHEMO-TEST	SURGICAL DENERVATION	CYCLOHEX	I	HEX	HYO	ACTH	C.A. EST.	HYD. EST.	PG. EST.
	P.	C/U										
1	+						+	(+)		+		
2	+						+			+		
3	+						(+)	+		+		
4	+						(+)	+		+		
5	+							+		+		
6		+					+	(+)		+		
7		+						+		+		
8		+								+		
9		+					+			+		
10		+				+		+		+		
11		+				+	+			+		
12		+				+		+		+		
13		+				+	+			+		
14		+				+	+			+		
15		+				+	+			+		
16		+				+	(+)	+		+		
17		+				+	+			+		
18		+	+			+			+	+	+	
19		+	+			(+)			+	+	+	
20		+				(+)				+		+
21		+							+	+	+	
22		+	+						+	+	+	
23		+	+	FAILED					+	+	+	
24		+						+	+	+	+	
25		+					+	+	+	+	+	
26		+	+	FAILED			+	+	+	+	+	
27		+	+	+						+	+	
28		+	+	+						+	+	
29		+	+	+						+	+	
30		+	+	+	+					+		
31		+	+	+	+					+		
32		+	+	FAILED	+					+		
33		+	+	FAILED						+	+	
34		+										

DOG 1 (10.9kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	220	31.5	7.46	135	69	53.08	9	-	1.25
2	3.5	BR 1	-	-	-	93	86	66.64	13	-	1.00
3	10	CONTROL	195	28.0	7.42	135	74	26.11	0	-	0.35
4	12.5	CR 1	30.5	47.0	7.16	135	90	12.28	9	-	0.18
5	22	CONTROL	192	31.5	7.33	134	69	21.50	40	-	1.90
6	24.5	BR 2	-	-	-	92	96	31.52	23	-	2.00
7	31	CONTROL	200	34.0	7.33	135	73	25.70	27	-	1.55
8	33.5	CR 2	44.5	42.0	7.18	135	82	31.92	7	-	1.25
9	42	CONTROL	195	32.0	7.34	135	58	21.79	0	-	1.45
10	44.5	BR 3	-	-	-	92	87	79.27	20	-	1.50
11	51	CONTROL	198	33.0	7.32	135	78	50.04	22	-	2.25
12	53.5	CR 3	31	48.5	7.06	135	104	22.57	14	-	2.80
	59	HEXAMETHONIUM (2mg/kg)									
13	71	CONTROL	202	31.0	7.34	135	68	9.25	64	-	2.50
14	73.5	BR 4	-	-	-	92	89	14.25	39	-	2.25
15	80	CONTROL	-	-	-	135	59	14.69	36	-	1.80
16	82.5	CR 4	15	50.0	7.06	135	74	11.94	42	-	1.60
17	91	CONTROL	168	30.0	7.32	134	54	21.32	55	-	1.50

DOG 1 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	93.5	BR 5	-	-	-	92	75	25.94	46	-	1.35
19	100	CONTROL	208	29.5	7.36	134	40	24.00	17	-	1.00
20	102.5	CR 5	22	44.0	7.13	134	60	13.18	26	-	0.85
	112	HYOSCINE METHYL BROMIDE (10mg/kg) + HEXAMETHONIUM (2mg/kg)									
21	123	CONTROL	200	24.0	7.30	134	47	2.46	10	-	0.70
22	125.5	BR 6	-	-	-	92	54	3.50	33	-	0.50
23	132	CONTROL	205	29.0	7.30	134	44	9.31	25	-	0.85
24	134.5	CR 6	8	53.0	6.98	134	51	5.32	40	-	0.50

DOG 2 (11.7kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	178	30.0	7.39	107	61	9.94	18	-	2.15
2	3.5	BR 1	-	-	-	82	105	25.13	23	-	3.50
3	10	CONTROL	170	29.0	7.43	105	68	10.51	24	-	2.05
4	12.5	CR 1	8	70.0	6.94	105	144	58.33	17	-	3.20
5	14.5	CR 1'	-	-	-	105	100	39.46	30	-	3.20
6	20	CONTROL	155	26.0	7.41	105	40	6.32	5	-	1.05
7	22.5	BR 2	-	-	-	79	92	15.47	23	-	1.65
8	29	CONTROL	178	28.0	7.37	105	34	8.16	17	-	1.00
9	31.5	CR 2	24.5	49.0	7.05	105	50	15.56	15	-	0.90
10	33.5	CR 2'	-	-	-	105	36	14.53	10	-	0.85
	36	HEXAMETHONIUM (2mg/kg)									
11	49	CONTROL	188	26.0	7.35	105	24	3.03	37	-	0.65
12	51.5	BR 3	-	-	-	80	29	7.52	26	-	0.75
13	58	CONTROL	178	25.5	7.33	105	25	4.19	24	-	0.80
14	60.5	CR 3	30	40.0	7.10	105	30	4.79	26	-	0.70
15	62.5	CR 3'	-	-	-	105	25	9.36	27	-	0.55

DOG 3 (12.0kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	115	51.0	7.16	127	54	5.73	41	-	2.40
2	3.5	BR 1	-	-	-	87	181	32.45	33	-	6.00
3	10	CONTROL	159	32.0	7.36	125	56	5.52	36	-	2.00
4	12.5	CR 1	34	37.0	7.26	125	63	1.28	24	-	2.00
5	14.5	CR 1'	-	-	-	125	47	2.96	24	-	1.80
6	20	CONTROL	140	37.0	7.29	124	45	6.06	18	-	1.40
7	22.5	BR 2	-	-	-	94	109	20.35	32	-	2.75
8	28.5	CONTROL	146	41.0	7.25	122	32	6.95	22	-	1.20
9	31	CR 2	25	44.0	7.14	122	37	2.33	30	-	1.05
10	33	CR 2'	-	-	-	122	34	2.21	37	-	1.25
	37	HYOSCINE METHYL BROMIDE (10mg/kg)									
11	53	CONTROL	145	42.0	7.19	121	39	1.16	26	-	1.50
12	55.5	BR 3	-	-	-	92	80	2.96	15	-	3.25
13	62	CONTROL	154	41.5	7.34	121	62	1.75	30	-	2.65
14	64.5	CR 3	21.5	49.5	7.15	121	64	0.29	-	-	2.75
15	66.5	CR 3'	-	-	-	121	50	0.52	-	-	2.55
16	73	CONTROL	170	34.0	7.42	122	54	0.30	-	-	2.10
17	75.5	BR 4	-	-	-	92	94	0.70	-	-	2.65

DOG 3 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	82	CONTROL	182	25.0	7.46	121	30	0.46	-	-	0.75
19	84.5	CR 4	23.5	41.5	7.17	121	44	0.94	-	-	0.45
20	86.5	CR 4'	-	-	-	121	25	0.63	-	-	0.55
	88	HEXAMETHONIUM (2mg/kg)									
21	102	CONTROL	172	25.0	7.42	120	31	0.13	-	-	0.65
22	104.5	BR 5	-	-	-	92	34	0.09	-	-	1.05
23	111	CONTROL	190	26.0	7.41	120	26	0.05	-	-	0.60
24	113.5	CR 5	28.5	35.5	7.18	120	26	0.26	-	-	0.55
25	115.5	CR 5'	-	-	-	120	24	0.23	-	-	0.43

DOG 4 (12.0kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	155	45.0	7.24	125	48	1.10	5	-	0.45
2	3.5	BR 1	-	-	-	95	92	11.05	5	-	0.85
3	40	CONTROL	129	40.0	7.27	121	38	8.90	2	-	1.90
4	42.5	CR 1	26.5	65.0	6.98	121	43	3.71	16	-	1.40
5	60	CONTROL	129	37.5	7.25	120	31	6.15	10	-	1.10
6	62.5	BR 2	-	-	-	92	51	29.06	6	-	1.40
7	71	CONTROL	143	33.0	7.25	120	29	6.56	6	-	0.85
8	73.5	CR 2	31	49.0	6.98	120	41	5.12	10	-	0.80
	78	HYOSCINE METHYL BROMIDE (10mg/kg)									
9	99	CONTROL	155	35.0	7.26	120	53	3.96	11	-	1.80
10	101.5	BR 3	-	-	-	91	79	12.44	9	-	1.90
11	108	CONTROL	170	31.0	7.29	120	48	5.31	11	-	1.60
12	110.5	CR 3	6.5	70.0	6.88	120	65	6.97	10	-	1.35
13	124	CONTROL	170	32.0	7.31	120	45	6.38	8	-	1.30
14	126.5	BR 4	-	-	-	93	70	17.90	10	-	1.45
15	133	CONTROL	192	29.0	7.30	119	38	9.65	6	-	1.40
16	135.5	CR 4	26.5	54.0	6.99	119	61	7.05	8	-	1.25
	141	HEXAMETHONIUM (2mg/kg)									

DOG 4 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
17	156	CONTROL	180	28.0	7.39	118	20	1.20	-	-	0.63
18	158.5	BR 5	-	-	-	90	21	2.80	-	-	0.60
19	164	CONTROL	192	27.0	7.38	118	13	2.34	-	-	0.43
20	166.5	CR 5	7.5	65.0	6.94	30	14	38.38	-	-	0.40

DOG 5 (10.1kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	205	38.0	7.37	121	70	1.56	-	-	0.70
2	3.5	BR 1	-	-	-	103	79	2.78	-	-	0.70
3	10	CONTROL	230	43.0	7.36	121	63	4.11	-	-	0.80
4	12.5	CR 1	55	46.0	7.30	121	76	2.17	-	-	0.90
5	27	CONTROL	175	35.5	7.36	120	58	2.88	-	-	0.50
6	29.5	BR 2	-	-	-	91	85	5.24	-	-	0.60
7	36	CONTROL	190	36.0	7.40	119	50	2.85	-	-	0.40
8	38.5	CR 2	39.5	51.0	7.17	119	82	2.71	-	-	0.90
	48					HYOSCINE METHYL BROMIDE (10mg/kg)					
9	63	CONTROL	175	34.0	7.40	119	56	0.85	-	-	0.55
10	65.5	BR 3	-	-	-	90	80	2.20	-	-	1.00
11	72	CONTROL	185	35.0	7.39	118	53	1.83	-	-	0.50
12	74.5	CR 3	5.5	66.0	6.10	110	70	1.07	-	-	0.35

DOG 6 (11.5kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	77	38.0	7.40	132	60	8.52	14	-	2.25
2	3.5	BR 1	-	-	-	99	109	17.64	9	-	2.75
3	10	CONTROL	205	36.0	7.40	129	49	12.04	5	-	1.40
4	12.5	CR 1	41	46.5	7.16	129	106	28.71	30	-	2.65
5	21	CONTROL	156	33.5	7.40	129	64	19.64	28	-	1.60
6	23.5	BR 2	-	-	-	99	120	35.96	39	-	2.35
7	30	CONTROL	168	30.5	7.42	128	57	31.08	28	-	1.55
8	32.5	CR 2	37	47.0	7.11	128	141	68.20	31	-	3.25
	35	HEXAMETHONIUM (10mg/kg)									
9	51	CONTROL	141	32.0	7.39	127	56	6.79	70	-	1.75
10	53.5	BR 3	-	-	-	98	67	7.33	30	-	1.85
11	60	CONTROL	149	32.0	7.36	127	48	3.37	10	-	1.35
12	62.5	CR 3	34	49.0	7.08	126	70	7.45	9	-	1.95
13	69	CONTROL	130	30.5	7.34	126	48	6.90	20	-	1.20
14	71.5	BR 4	-	-	-	96	65	7.69	20	-	1.40
15	78.25	CONTROL	146	32.0	7.34	126	41	5.29	37	-	0.92
16	80.75	CR 4	41	42.0	7.12	119	65	9.23	23	-	1.70
	84	HYOSCINE METHYL BROMIDE (10mg/kg)									

DOG 6 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
17	98	CONTROL	134	34.0	7.30	125	40	2.58	11	-	0.80
18	100.5	BR 5	-	-	-	95	44	2.55	12	-	0.90
19	107	CONTROL	144	34.5	7.29	125	39	2.53	22	-	0.70
20	109.5	CR 5	38	56.0	7.01	119	47	1.79	10	-	0.90

DOG 7 (9.0kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	77	35.0	7.48	-	-	18.44	29	-	1.85
2	3.5	BR 1	-	-	-	-	-	21.15	14	-	2.75
3	10	CONTROL	76	34.0	7.47	123	42	7.71	14	-	1.10
4	12.5	CR 1	35	55.0	7.20	123	162	21.50	6	-	3.00
5	26	CONTROL	195	35.5	7.41	124	42	3.05	16	-	1.25
6	28.5	BR 2	-	-	-	94	50	4.94	10	-	1.30
7	35	CONTROL	220	34.0	7.40	124	53	19.55	10	-	2.45
8	37.5	CR 2	8	89.0	6.90	115	113	38.53	11	-	3.25
9	44	CONTROL	215	35.5	7.39	123	44	9.68	10	-	1.40
10	46.5	BR 3	-	-	-	84	60	12.23	12	-	1.15
	48					HYOSCINE METHYL BROMIDE (10mg/kg)					
11	65	CONTROL	175	37.0	7.36	122	51	6.35	15	-	2.00
12	67.5	BR 4	-	-	-	83	67	8.48	10	-	2.15
13	74	CONTROL	185	36.0	7.40	122	36	3.37	10	-	1.05
14	76.5	CR 3	40	45.0	7.20	122	91	3.86	18	-	1.35
15	84	CONTROL	150	35.5	7.34	122	39	3.80	17	-	1.05
16	86.5	BR 5	-	-	-	83	56	5.32	17	-	1.25
17	94	CONTROL	185	34.0	7.36	121	49	5.61	18	-	2.15
18	96.5	CR 4	31	55.0	7.12	121	110	5.02	15	-	2.15

DOG 8 (19.0kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	190	-	7.33	108	49	3.07	60	-	3.25
2	3.5	BR 1	-	-	-	72	90	4.84	30	-	4.00
3	10	CONTROL	205	-	7.31	108	42	4.32	60	-	2.15
4	12.5	CR 1	20.5	-	7.21	110	76	3.48	40	-	3.70
5	20	CONTROL	250	47.0	7.32	112	38	3.73	54	-	1.30
6	22.5	BR 2	-	-	-	73	82	13.10	73	-	2.50
7	29	CONTROL	212	47.0	7.32	111	31	3.44	46	-	0.65
						DAMAGED GLAND - NO FURTHER RELEASE					

DOG 9 (10.5kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	112	33.0	7.34	120	77	28.43	20	-	2.75
2	3.5	BR 1	-	-	-	92	115	35.45	18	-	3.75
3	10	CONTROL	109	36.5	7.34	120	61	24.13	15	-	2.25
4	12.5	CR 1	36	50.0	7.12	120	73	18.90	15	-	2.25
5	21	CONTROL	135	33.5	7.34	120	63	19.82	22	-	2.60
6	23.5	BR 2	-	-	-	93	105	33.61	20	-	3.85
7	30	CONTROL	142	35.5	7.33	120	65	20.83	16	-	3.15
8	32.5	CR 2	3.5	58.0	6.92	117	95	22.03	14	-	3.75
	38					HEXAMETHONIUM (10mg/kg)					
9	51	CONTROL	165	-	7.32	118	60	0.43	-	-	2.65
10	53.5	BR 3	-	-	-	82	80	0.17	-	-	3.70
11	60	CONTROL	177	30.0	7.37	118	66	0.00	-	-	3.15
12	62.5	CR 3	0.5	60.0	7.02	118	104	0.80	-	-	4.25
13	74	CONTROL	174	30.0	7.39	117	53	0.16	-	-	2.25
14	76.5	BR 4	-	-	-	90	69	0.16	-	-	2.80
15	83	CONTROL	187	28.0	7.39	117	51	0.48	-	-	2.20
16	85.5	CR 4	14	45.0	7.14	102	62	0.64	-	-	2.05

DOG 10 (23.8kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	175	35.5	7.35	136	178	13.89	29	-	6.80
2	3.5	BR 1	-	-	-	105	300	23.61	79	-	5.75
3	10	CONTROL	160	35.0	7.35	136	122	4.52	37	-	3.95
4	12.5	CR 1	26.5	51.0	7.07	136	218	6.24	63	-	4.15
5	20	CONTROL	172	32.0	7.39	135	129	3.59	92	-	2.50
6	22.5	BR 2	-	-	-	101	235	9.31	92	-	3.25
7	29	CONTROL	202	31.0	7.39	135	102	1.62	25	-	1.70
8	31.5	CR 2	25.8	46.0	7.11	135	201	12.29	81	-	4.30
	38	HYOSCINE METHYL BROMIDE (10mg/kg)									
9	57	CONTROL	195	32.5	7.35	136	115	3.53	31	-	2.90
10	59.5	BR 3	-	-	-	101	173	5.36	25	-	2.90
11	66	CONTROL	202	32.5	7.36	135	100	5.50	30	-	1.90
12	68.5	CR 3	10	61.0	7.00	135	196	6.79	24	-	3.20
13	77	CONTROL	182	29.0	7.37	135	76	1.45	24	-	0.95
14	87	CONTROL	175	31.0	7.36	135	58	1.55	33	-	0.50
15	89.5	CR 4	39	42.0	7.16	135	150	10.29	44	-	1.75
16	96	CONTROL	188	35.0	7.40	135	105	5.15	100	-	2.60
17	98.5	BR 4	-	-	-	103	183	7.21	100	-	2.65

DOG 10 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	105	CONTROL	205	30.5	7.37	134	70	3.26	34	-	1.25
19	107.5	CR 5	25.5	51.0	7.09	134	175	8.91	40	-	2.70

DOG 11 (20.0kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	180	34.5	7.41	116	70	3.23	20	-	0.85
2	3.5	BR 1	-	-	-	80	153	9.45	30	-	1.15
3	10	CONTROL	190	33.0	7.40	106	91	5.83	46	-	0.85
4	12.5	CR 1	29	50.0	7.18	106	170	20.42	61	-	1.30
5	24	CONTROL	195	29.0	7.41	105	83	9.10	67	-	0.50
6	26.5	BR 2	-	-	-	69	144	10.00	79	-	0.45
7	36	CONTROL	200	28.0	7.39	106	54	9.79	42	-	0.60
8	38.5	CR 2	32	46.0	7.11	106	151	26.36	90	-	2.15
	42						HEXAMETHONIUM (10mg/kg)				
9	59	CONTROL	202	29.5	7.48	105	50	0.28	-	-	1.25
10	61.5	BR 3	-	-	-	67	55	1.17	-	-	1.15
11	69	CONTROL	218	26.0	7.50	105	39	1.10	-	-	0.75
12	71.5	CR 3	29	41.0	7.28	105	63	3.54	70	-	1.60
13	80	CONTROL	215	26.0	7.47	105	31	2.43	75	-	0.70
14	82.5	BR 4	-	-	-	68	40	3.27	75	-	0.85
15	90	CONTROL	212	25.0	7.47	105	24	3.09	40	-	0.50
16	92.5	CR 4	27	40.5	7.26	105	52	5.77	76	-	0.75

DOG 12 (11.1kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	192	41.5	7.36	123	99	3.93	16	-	2.90
2	3.5	BR 1	-	-	-	87	174	9.54	30	-	3.15
3	10	CONTROL	215	36.5	7.33	122	90	5.15	44	-	2.50
4	12.5	CR 1	51	47.0	7.23	122	128	5.40	20	-	1.85
5	19	CONTROL	202	34.0	7.36	122	84	2.12	18	-	1.65
6	21.5	CR 2	35.5	51.0	7.12	122	147	6.56	46	-	1.75
7	29	CONTROL	118	37.0	7.32	123	75	2.25	18	-	1.15
8	31.5	BR 2	-	-	-	90	151	6.69	26	-	1.75
9	38	CONTROL	180	32.0	7.30	123	57	1.41	30	-	1.25
10	40.5	CR 3	38.5	48.0	7.16	123	110	5.63	25	-	1.95
11	42.5	CR 3'	-	-	-	123	92	2.77	20	-	1.75
	51	HYOSCINE METHYL BROMIDE (10mg/kg)									
12	66	CONTROL	208	39.0	7.35	123	85	0.93	10	-	2.45
13	68.5	CR 4	4	79.0	6.97	123	114	2.32	10	-	2.30
14	75	CONTROL	106	44.0	7.32	121	71	1.10	30	-	1.50
15	77.5	BR 3	-	-	-	83	108	4.96	47	-	1.65
16	86	CONTROL	195	39.0	7.33	121	65	1.32	20	-	1.55
17	88.5	CR 5	23	66.0	7.07	121	100	2.09	16	-	1.75

DOG 12 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	95	CONTROL	170	39.0	7.34	121	65	1.35	30	-	1.65
19	98.5	BR 4	-	-	-	81	101	3.75	36	-	1.75
	100	HYOSCINE METHYL BROMIDE (10mg/kg)									
20	119	CONTROL	238	40.0	7.34	119	97	1.07	10	-	2.45
21	121.5	CR 6	44	63.0	7.12	119	130	2.74	6	-	2.75
22	129	CONTROL	190	37.5	7.31	119	99	1.23	6	-	2.10
23	131.5	BR 5	-	-	-	83	150	4.16	6	-	2.70
24	139	CONTROL	228	40.0	7.31	119	84	0.68	-	-	2.00
25	141.5	CR 7	28	66.0	7.04	119	114	3.56	15	-	1.80
26	148	CONTROL	210	37.0	7.32	119	63	1.74	15	-	1.25
27	150.5	BR 6	-	-	-	83	102	4.34	15	-	1.80

DOG 13 (23.0kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	210	39.0	7.36	118	92	0.68	5	-	3.45
2	3.5	BR 1	-	-	-	83	218	3.72	0	-	4.40
3	10	CONTROL	208	39.0	7.37	118	69	0.49	8	-	2.60
4	12.5	CR 1	41	52.0	7.16	118	165	3.51	6	-	3.65
5	20	CONTROL	195	35.0	7.39	118	70	0.92	15	-	2.25
6	22.5	BR 2	-	-	-	83	217	7.08	20	-	3.75
7	29	CONTROL	235	33.0	7.42	118	85	2.52	8	-	2.65
8	31.5	CR 2	15	63.0	7.04	118	194	19.15	23	-	4.50
	35	HEXAMETHONIUM (10mg/kg)									
9	56	CONTROL	220	39.0	7.30	115	79	0.26	-	-	3.45
10	58.5	BR 3	-	-	-	79	138	0.87	-	-	4.80
11	65	CONTROL	240	35.5	7.34	114	78	0.09	-	-	2.85
12	67.5	CR 3	33	62.0	7.03	114	139	1.09	-	-	3.65
13	76	CONTROL	240	32.0	7.37	113	62	0.08	-	-	1.90
14	78.5	BR 4	-	-	-	78	144	1.11	-	-	3.05
15	85	CONTROL	255	33.0	7.38	113	52	0.11	-	-	1.40
16	87.5	CR 4	27	70.0	7.02	113	127	2.43	30	-	3.05

DOG 14 (9.7kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	207	36.0	7.37	125	79	10.45	25	-	2.50
2	3.5	BR 1	-	-	-	87	130	10.62	30	-	2.90
3	10	CONTROL	230	40.0	7.38	126	81	7.41	9	-	2.45
4	12.5	CR 1	44	46.0	7.24	126	113	23.40	32	-	2.25
5	20	CONTROL	185	36.0	7.36	125	105	4.98	6	-	2.55
6	22.5	BR 2	-	-	-	86	162	6.86	3	-	2.85
7	29	CONTROL	210	40.0	7.37	124	80	5.16	5	-	1.90
8	31.5	CR 2	32	52.0	7.14	124	107	18.23	31	-	2.00
	33						HEXAMETHONIUM (10mg/kg)				
9	54	CONTROL	218	36.0	7.42	124	67	0.93	-	-	2.40
10	56.5	BR 3	-	-	-	85	112	0.59	-	-	2.40
11	63	CONTROL	212	-	-	123	70	0.14	-	-	1.85
12	65.5	CR 3	22.5	63.0	7.10	123	113	1.03	-	-	1.85
13	77	CONTROL	206	34.0	7.42	121	80	0.58	-	-	1.50
14	79.5	BR 4	-	-	-	83	97	1.24	-	-	1.60
15	86	CONTROL	225	34.0	7.43	122	56	0.63	-	-	1.20
16	88.5	CR 4	3.5	66.0	7.04	122	66	1.03	-	-	0.80
17	96	CONTROL	200	34.0	7.41	121	93	0.76	-	-	1.60

DOG 14 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	98.5	CR 5	36.5	53.0	7.16	121	102	1.86	-	-	1.40
19	108	CONTROL	210	30.0	7.36	121	87	0.34	-	-	1.50
20	110.5	BR 5	-	-	-	84	98	0.24	-	-	1.50

DOG 15 (11.0kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	202	41.5	7.37	130	-	38.33	17	-	4.65
2	3.5	BR 1	-	-	-	115	-	56.70	19	-	4.80
3	10	CONTROL	198	38.0	7.40	130	-	72.79	3	-	3.40
4	12.5	CR 1	24.5	72.0	7.07	130	-	78.05	5	-	2.90
5	22	CONTROL	182	38.0	7.38	130	-	63.00	5	-	3.10
6	24.5	BR 2	-	-	-	100	-	91.65	6	-	3.65
7	31	CONTROL	190	40.0	7.35	130	-	55.98	4	-	2.50
8	33.5	CR 2	2	84.0	6.90	130	-	45.13	3	-	2.45
	36	HEXAMETHONIUM (10mg/kg)									
9	58	CONTROL	195	40.0	7.36	130	-	2.16	-	-	2.10
10	60.5	BR 3	-	-	-	90	-	5.58	-	-	2.40
11	67	CONTROL	195	41.5	7.36	130	-	4.43	-	-	1.50
12	69.5	CR 3	28	55.0	7.14	130	-	3.07	-	-	1.65
13	90	CONTROL	192	40.5	7.36	130	-	7.31	-	-	3.65
14	92.5	BR 4	-	-	-	90	-	7.26	-	-	3.75
15	99	CONTROL	195	39.0	7.36	130	-	2.79	-	-	3.60
16	101.5	CR 4	3	79.0	6.97	130	-	4.45	-	-	3.15

DOG 16 (11.0kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	112	37.0	-	140	82	2.03	32	-	1.40
2	3.5	BR 1	-	-	-	102	126	3.51	24	-	1.50
3	10	CONTROL	115	35.0	-	140	60	2.91	23	-	0.90
4	12.5	CR 1	46	44.0	-	140	79	5.00	-	-	0.85
5	19	CONTROL	101	36.0	-	140	99	4.58	0	-	0.90
6	21.5	CR 2	35	43.0	-	140	146	3.87	8	-	1.05
7	29	CONTROL	117	33.5	-	140	136	6.69	7	-	1.10
8	31.5	BR 2	-	-	-	111	213	8.51	9	-	1.40
9	38	CONTROL	125	31.0	-	140	122	3.37	10	-	0.90
10	40.5	CR 3	26	48.0	-	140	177	5.68	-	-	0.15
11	49	CONTROL	120	29.0	-	140	126	4.55	9	-	0.85
12	51.5	BR 3	-	-	-	123	171	7.65	9	-	1.15
13	58	CONTROL	120	29.0	-	140	98	4.55	-	-	1.00
14	60.5	CR 4	21	54.0	-	140	184	7.97	11	-	1.35
	63	HYOSCINE METHYL BROMIDE (10mg/kg)									
15	89	CONTROL	104	34.0	-	141	121	3.30	10	-	1.20
16	91.5	BR 4	-	-	-	116	156	4.58	10	-	1.25
17	98	CONTROL	114	30.0	-	141	112	2.80	7	-	0.90

DOG 16 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	100.5	CR 5	22	47.0	-	141	181	4.16	16	-	1.10
	106					HYOSCINE METHYL BROMIDE (10mg/kg)					
19	129	CONTROL	90	37.0	-	141	124	3.23	-	-	1.10
20	131.5	BR 5	-	-	-	112	161	4.09	19	-	1.20
21	138	CONTROL	106	32.0	-	141	104	3.30	24	-	0.90
22	140.5	CR 6	27	47.0	-	141	156	4.09	-	-	1.05
23	152	CONTROL	-	-	-	141	96	4.01	21	-	0.90
24	154.5	BR 6	-	-	-	112	137	4.58	19	-	1.00
	156					HEXAMETHONIUM (10mg/kg)					
25	164	CONTROL	132	30.0	-	140	61	0.07	-	-	0.65
26	166.5	BR 7	-	-	-	101	63	0.02	-	-	0.60
27	171	CONTROL	131	30.0	-	140	90	0.20	-	-	0.73
28	173.5	CR 7	29	47.0	-	140	90	0.20	-	-	1.00

DOG 17 (13.2kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	127	34.0	7.43	145	100	1.17	-	-	2.50
2	3.5	BR 1	-	-	-	110	151	2.05	21	-	2.65
3	10	CONTROL	123	33.0	7.57	145	74	1.50	-	-	1.65
4	12.5	CR 1	37	39.0	7.31	145	78	0.99	-	-	1.50
5	21	CONTROL	116	37.0	7.29	147	78	2.59	23	-	1.45
6	23.5	CR 2	35	43.0	7.18	147	122	2.11	27	-	1.50
7	32	CONTROL	103	39.5	7.35	147	87	1.74	-	-	1.65
8	34.5	BR 2	-	-	-	118	154	4.03	24	-	2.30
9	41	CONTROL	112	41.0	7.34	147	75	2.11	18	-	1.45
10	43.5	CR 3	24.5	44.5	7.15	147	144	3.01	20	-	1.70
	48	HEXAMETHONIUM (2mg/kg)									
11	57	CONTROL	116	34.5	7.36	146	82	0.31	-	-	2.20
12	59.5	BR 3	-	-	-	110	105	0.50	-	-	2.20
13	66	CONTROL	114	43.0	7.36	147	84	0.16	-	-	2.15
14	68.5	CR 4	23	56.0	7.16	147	96	0.48	-	-	1.95
15	76	CONTROL	109	38.0	7.37	147	70	0.35	-	-	1.30
16	78.5	BR 4	-	-	-	112	112	0.41	-	-	1.55
17	85	CONTROL	111	41.0	7.35	147	74	0.04	-	-	1.00

DOG 17 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	87.5	CR 5	27	59.0	7.17	147	91	0.55	-	-	1.40
	90					HEXAMETHONIUM (8mg/kg)					
19	100	CONTROL	104	44.0	7.31	147	87	0.07	-	-	1.80
20	102.5	BR 5	-	-	-	108	101	0.13	-	-	1.80
21	109	CONTROL	185	42.0	7.35	147	80	0.10	-	-	1.35
22	111.5	CR 6	27	52.0	7.20	147	67	0.25	-	-	1.25
23	121	CONTROL	106	41.0	7.31	147	67	0.12	-	-	1.05
24	123.5	BR 6	-	-	-	108	86	0.18	-	-	1.20
25	130	CONTROL	116	43.0	7.36	147	76	0.10	-	-	1.25
26	132.5	CR 7	23	45.0	7.13	147	87	0.07	-	-	1.35
27	140	CONTROL	102	40.0	7.31	146	70	0.14	-	-	1.00
28	142.5	BR 7	-	-	-	109	100	0.39	-	-	1.30
29	149	CONTROL	117	41.0	7.32	146	55	0.27	-	-	0.85
30	151.5	CR 8	24	41.0	7.21	146	70	0.29	-	-	1.00

DOG 18 (13.1kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	108	43.0	7.36	140	67	6.44	32	1.817	3.10
2	3.5	BR 1	-	-	-	105	160	-	-	1.802	3.50
3	15	CONTROL	-	-	-	140	73	9.73	31	1.733	2.05
4	17.5	BR 2	-	-	-	112	177	9.86	19	1.779	3.55
	21	DENERVATION (LIGNOCAINE + CRUSHING)									
5	36	CONTROL	118	39.0	7.32	140	83	3.41	23	2.046	3.20
6	38.5	BR 3	-	-	-	105	127	-	-	1.954	3.70
7	45	CONTROL	-	-	-	140	93	-	-	1.817	2.30
8	47.5	BR 4	-	-	-	105	147	1.99	40	1.405	2.75
9	55	CONTROL	126	35.0	7.39	140	83	1.78	-	0.420	1.85
	56	LONG CHEMOTEST (10 MINUTES)									
10	57.5	LCR	86	38.0	7.33	140	70	0.68	-	0.504	2.43
	59	LCR	48	37.0	7.30						
	62.5	LCR	46.5	40.0	7.24						
11	66	LCR	47	39.0	7.24	140	107	-	-	1.672	2.20
	70	-	121	30.5	7.35						
12	76.5	-	-	-	-	140	127	-	-	1.771	1.47
13	79	-	-	-	-	140	147	1.95	-	1.504	1.75

DOG 18 (Contd.)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	ADRENAL OUTPUT			
			pO_2	pCO_2	pH			C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
14	87.5	-	-	-	-	140	117	0.73	-	2.244	1.77
15	90.5	-	-	-	-	140	83	-	-	2.344	1.50
16	93.5	BR 5	-	-	-	112	150	2.08	39	1.427	1.80
17	99	-	116	33.0	7.39	140	90	-	-	2.176	1.50
18	107	-	-	-	-	140	77	-	-	1.679	1.47
19	115	-	-	-	-	140	153	1.65	-	2.282	2.40
20	118	BR 6	-	-	-	124	177	11.41	25	1.344	1.75
	120	ACTH (25µg/min for 10 minutes)									
21	129.5	ACTH	-	-	-	140	100	4.60	-	2.893	3.13
22	139.5	-	-	-	-	140	77	15.12	30	1.763	1.73
23	149.5	-	-	-	-	140	50	17.79	25	1.672	1.33
24	159.5	-	113	32.0	7.36	140	60	19.88	31	2.198	1.90

DOG 19 (12.5kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$	FLOW (ml/min)
1	1	CONTROL	165	33.0	7.40	132	68	7.68	18	1.584	3.45
2	3.5	BR 1	-	-	-	100	155	14.39	20	1.656	4.75
3	10	CONTROL	-	-	-	132	66	14.24	20	1.976	3.20
4	12.5	BR 2	-	-	-	102	132	30.00	20	2.120	4.15
5	19	CONTROL	-	-	-	132	64	24.16	27	2.520	3.50
6	21.5	BR 3	-	-	-	102	123	29.05	22	2.176	5.00
	45	DENERVATION (LIGNOCAINE + CRUSHING) + INDOMETHACIN (5mg/kg)									
7	61	CONTROL	155	32.0	7.34	132	106	4.67	11	2.352	1.75
8	63.5	BR 4	-	-	-	113	130	5.37	-	1.808	2.20
9	70	CONTROL	-	-	-	132	91	3.02	22	2.056	1.75
10	72.5	BR 5	-	-	-	111	136	4.00	24	2.224	2.25
11	78.5	CONTROL	165	34.0	7.33	132	91	1.73	30	2.248	1.67
	80	LONG CHEMOTEST (10 MINUTES)									
	81	LCR	2	66.0	6.94						
12	82.5	LCR	45	37.0	7.22	132	138	1.39	-	2.008	1.57
	86	LCR	44	35.0	7.20						
	88	LCR	45.5	37.5	7.20						
13	91.5	-	-	-	-	132	104	0.94	-	2.000	1.00

DOG 19 (cont'd)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$	FLOW (ml/min)
14	95	BR 6	-	-	-	85	183	5.22	-	2.368	2.35
15	101.5	-	-	-	-	132	100	0.99	-	2.288	1.67
16	111.5	-	-	-	-	132	89	0.74	-	2.160	1.53
17	115.5	BR 7	-	-	-	113	134	0.52	-	2.024	1.50
18	121.5	-	170	-	7.37	132	85	0.64	-	2.080	1.67
19	131.5	-	-	-	-	132	72	1.58	-	2.312	1.47
20	135.5	BR 8	-	-	-	115	147	1.94	-	2.280	2.45
21	141.5	-	143	-	7.45	132	91	6.50	31	2.152	2.27
	146	ACTH (25µg/min for 10 minutes)									
22	156	ACTH	-	-	-	132	91	47.32	33	2.296	7.07
23	166	-	-	-	-	132	74	38.52	-	2.496	4.30
24	170.5	BR 9	-	-	-	104	157	81.78	21	2.144	5.00
25	176	-	170	25.0	7.41	132	85	12.09	18	2.584	4.20
26	186	-	-	-	-	132	64	19.75	26	2.688	3.65
27	196	-	177	23.0	7.48	132	57	40.38	21	2.52	3.50

DOG 20 (11.6kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	FLOW (ml/min)	6-oxo-$PGF_{1\alpha}$ ($ng.kg^{-1}min^{-1}$)
1	1.5	CONTROL	170	21.0	7.42	130	55	21.6	21	2.93	0.491
2	4.75	BR 1	-	-	-	95	102	33.2	20	4.04	0.742
3	15.5	CONTROL	200	20.0	7.31	130	69	18.1	18	3.30	0.520
4	18.75	BR 2	-	-	-	97	116	27.7	17	3.96	0.697
5	24.5	CONTROL	-	-	-	130	66	18.0	23	2.50	0.491
6	27.75	BR 3	-	-	-	96	124	31.2	20	3.72	0.671
	30.5					INDOMETHACIN (5mg/kg)					
7	39.5	CONTROL	128	27.0	7.28	135	91	20.5	20	3.07	0.430
8	42.75	BR 4	-	-	-	96	113	36.1	18	3.00	0.386
9	48.5	CONTROL	195	25.0	7.31	135	97	16.0	18	2.63	0.381
10	50.75	BR 5	-	-	-	100	137	34.3	22	3.36	0.495
11	61.5	CONTROL	-	-	-	136	94	12.2	20	1.92	0.263
12	65.75	BR 6	-	-	-	108	142	31.0	21	3.08	0.289
	71					GUANETHIDINE (2mg/kg)					
13	86.5	CONTROL	-	-	-	135	59	13.1	17	1.50	0.172
14	90	BR 7	-	-	-	97	78	23.0	14	1.43	0.136
	97					GUANETHIDINE (2mg/kg)					
15	106.5	CONTROL	200	19.0	7.34	137	49	12.2	22	1.10	0.087

DOG 20 (cont'd)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$	NA%	FLOW (ml/min)	6-oxo-$PGF_{1\alpha}$ ($ng.kg^{-1}min^{-1}$
16	110	BR 8	-	-	-	95	49	19.1	16	1.03	0.092
17	117.5	CONTROL	-	-	-	136	47	9.8	15	1.00	0.078
18	121	BR 9	-	-	-	96	47	21.2	15	1.23	0.097
19	128	CONTROL	-	-	-	136	42	11.1	17	0.98	0.103
20	132.5	BR 10	-	-	-	95	45	23.4	20	1.23	0.131

DOG 21 (17.8kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$	FLOW (ml/min)
1	1	CONTROL	59	36.0	7.26	-	-	43.64	26	0.972	5.08
2	4	CONTROL	-	-	-	-	-	49.12	24	1.051	5.15
	9					ACTH (25µg/min for 10 minutes)					
3	11	ACTH	103	36.0	7.29	-	-	111.08	28	1.331	6.25
4	21	-	-	-	-	-	-	93.66	21	0.719	1.40
5	30.5	-	-	-	-	-	-	67.20	19	1.107	10.16
6	35.5	-	-	-	-	-	-	130.69	25	1.011	7.30
7	41	-	-	-	-	-	-	118.55	31	1.028	3.50
8	45.5	-	-	-	-	-	-	50.05	27	1.067	8.00
9	50.5	-	-	-	-	-	-	76.49	25	1.163	6.80
10	56.5	-	-	-	-	-	-	96.75	36	0.713	2.08
11	65.5	-	-	-	-	-	-	60.98	30	1.449	6.30
12	71	-	52	32.0	7.16	-	-	126.06	21	0.933	4.33
13	118	-	-	-	-	-	-	47.23	40	0.944	3.00
14	121	-	-	-	-	-	-	85.28	18	0.730	2.40

DOG 22 (9.7kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$	FLOW (ml/min)
1	1	CONTROL	132	26.0	7.30	140	108	85.05	32	1.552	3.75
2	3.5	BR 1	-	-	-	102	164	123.35	29	1.505	4.50
3	10	CONTROL	133	34.0	7.30	140	86	93.34	27	1.495	3.35
4	12.5	BR 2	-	-	-	102	150	121.95	27	1.593	4.05
5	21	CONTROL	136	26.0	7.36	140	78	96.58	22	1.737	3.60
	22	LONG CHEMOTEST (18.5 minutes)									
6	24	LCR	43	38.0	7.19	140	69	-	-	1.804	3.75
7	26	LCR	40	40.0	7.17	140	83	106.07	20	1.500	3.50
8	28	LCR	45	35.0	7.21	140	89	-	-	1.814	3.45
9	30	LCR	-	-	-	140	92	147.28	23	1.557	3.30
10	32	LCR	48	33.0	7.19	133	92	-	-	1.768	3.65
11	34	LCR	46	34.0	7.17	120	92	274.16	28	1.593	4.20
12	36	LCR	46	28.0	7.16	107	75	-	-	1.526	3.25
13	41	LCR	45	32.0	7.11	93	58	229.57	28	1.314	2.25
14	46	-	122	25.0	7.26	140	36	-	-	0.866	1.15
15	51	-	-	-	-	140	28	93.67	27	1.000	1.15
16	56	-	162	24.0	7.21	140	36	-	-	1.794	2.30
17	61	-	-	-	-	140	67	90.72	29	2.196	3.50

DOG 22 (cont'd)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$	FLOW (ml/min)
18	66	-	156	24.0	7.27	140	92	-	-	2.335	3.45
19	71	-	-	-	-	140	94	110.83	32	1.366	2.60
20	76	-	-	-	-	140	83	-	-	1.892	2.30
21	81	-	-	-	-	140	75	116.42	30	1.711	2.00
22	93	CONTROL	-	-	-	140	67	141.42	36	1.227	1.40
	95		ACTH (6.25µg/min for 4 minutes)								
23	96	ACTH	-	-	-	140	86	-	-	2.773	3.05
24	98	ACTH	-	-	-	140	81	-	-	2.428	2.60
25	100	-	-	-	-	140	75	147.91	31	1.608	2.35
26	102	-	-	-	-	140	69	-	-	1.454	2.00
27	104	-	-	-	-	140	78	-	-	2.015	2.40
28	106	-	-	-	-	140	94	-	-	2.825	3.25
29	111	-	-	-	-	140	92	185.57	33	2.799	4.00
30	116	-	-	-	-	140	72	-	-	1.232	2.25
31	121	-	-	-	-	140	67	155.53	42	0.732	1.85
32	126	-	158	30.0	7.25	140	61	-	-	1.809	2.45
33	136	-	-	-	-	140	44	151.26	36	0.732	1.50
34	146	-	-	-	-	140	33	-	-	1.216	1.15
35	156	-	-	-	-	100	22	-	-	0.263	0.40

DOG 23 (12.0kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	113	27.5	7.37	140	81	29.67	20	1.667	3.00
2	3.5	BR 1	-	-	-	100	133	54.75	29	2.000	3.80
3	10	CONTROL	111	30.0	7.36	140	78	39.42	10	1.608	3.10
4	12.5	BR 2	-	-	-	100	133	69.00	17	1.700	3.90
5	21	CONTROL	106	28.0	7.26	140	78	30.75	11	1.783	4.50
	22	LONG CHEMOTEST (15 minutes)									
6	23	LCR	50	38.5	7.11	140	80	30.92	26	2.125	4.75
7	25	LCR	43	39.0	7.12	140	89	31.00	30	2.267	4.85
8	27	LCR	50	37.5	7.16	140	98	47.83	24	1.908	5.15
9	29	LCR	50.5	37.0	7.13	140	103	58.75	27	2.575	5.65
10	31	LCR	45	40.0	7.10	140	113	88.72	27	2.517	5.90
11	36	LCR	48	36.5	7.07	140	102	138.42	27	1.717	4.90
12	41	-	119	29.5	7.18	140	70	48.58	26	1.658	3.50
13	46	-	116	29.0	7.18	140	64	31.25	27	1.550	3.00
14	51	-	-	-	-	140	70	31.92	24	1.758	3.20
15	56	-	113	28.0	7.18	140	75	46.92	14	2.325	3.50
16	61	-	-	-	-	140	70	29.92	20	2.433	3.50
17	66	-	-	-	-	140	70	37.08	9	2.850	3.65

DOG 23 (cont'd)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	71	-	-	-	-	140	64	31.75	15	2.383	3.45
19	76	-	-	-	-	140	63	25.75	12	2.742	3.80
20	81	-	-	-	-	140	81	-	-	2.617	4.50
21	96	-	-	-	-	140	75	18.75	11	2.767	3.85
	97					ACTH (6.25µg/min for 4 minutes)					
22	98	ACTH	-	-	-	140	72	70.00	12	3.250	5.30
23	100	ACTH	-	-	-	140	83	123.25	10	3.675	6.80
24	102	-	-	-	-	140	73	60.75	11	2.550	6.40
25	104	-	-	-	-	140	69	39.83	10	2.692	6.10
26	106	-	-	-	-	140	64	42.08	8	2.300	5.00
27	111	-	111	24.0	7.21	140	56	41.92	7	2.250	4.15
28	116	-	-	-	-	140	52	49.83	8	2.200	3.90
29	121	-	-	-	-	140	72	-	-	2.275	5.50
30	126	-	-	-	-	140	75	35.08	10	2.375	5.55
31	136	-	-	-	-	140	69	20.08	12	2.533	5.30
32	146	-	-	-	-	140	67	25.83	9	2.292	5.20
33	156	-	107	22.0	7.22	140	69	33.92	6	1.917	5.25

DOG 24 (15.0kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	113	32.0	7.43	-	135	4.96	23	0.840	3.50
2	3	CONTROL	-	-	-	-	135	4.45	23	0.730	3.75
	5					ACTH (25µg/min for 1 minute)					
3	6	ACTH	83	34.0	7.34	-	131	6.45	10	0.973	5.25
4	8	-	-	-	-	-	125	7.93	7	0.900	6.25
5	10	-	-	-	-	-	122	8.09	6	0.893	5.25
6	12	-	-	-	-	-	124	8.73	6	0.920	4.85
7	14	-	-	-	-	-	118	9.22	10	1.087	4.50
8	16	-	-	-	-	-	118	10.71	2	1.193	4.55
9	21	-	-	-	-	-	114	12.04	1	1.140	3.90
10	26	-	-	-	-	-	120	7.52	17	1.340	4.05
11	31	-	-	-	-	-	127	15.77	8	1.587	4.00
12	43	-	135	18.5	7.30	-	108	22.62	12	1.367	3.25
13	55	-	-	-	-	-	108	32.38	4	1.527	3.30
	64					HYOSCINE METHYL BROMIDE (10mg/kg)					
14	79	CONTROL	184	32.5	7.31	-	98	11.35	-	1.433	2.80
	81					ACTH (25µg/min for 1 minute)					
15	82	ACTH	-	-	-	-	102	16.07	1	1.540	3.80
16	84	-	-	-	-	-	98	15.71	3	1.380	3.80
17	86	-	-	-	-	-	92	16.55	8	1.233	3.10

DOG 24 (cont'd)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	88	-	-	-	-	-	84	21.41	4	1.147	2.75
19	90	-	-	-	-	-	80	25.93	6	1.233	2.45
20	92	-	-	-	-	-	78	29.53	7	1.400	2.55
21	96	-	-	-	-	-	88	25.61	6	1.500	3.10
22	101	-	-	-	-	-	75	34.29	5	1.187	2.50
23	106	-	-	-	-	-	59	43.05	7	1.100	2.05
24	111	-	-	-	-	-	69	50.00	11	0.767	2.00

DOG 25 (16.5kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	115	38.0	7.32	142	90	11.19	22	0.388	4.15
2	4	CONTROL	-	-	-	142	86	12.27	15	0.406	4.45
	11.5	HYOSCINE METHYL BROMIDE (10mg/kg) + HEXAMETHONIUM BROMIDE (10mg/kg)									
3	28.5	CONTROL	-	-	-	142	57	0	-	0.321	3.25
	30	ACTH (25µg/min for 1 minute)									
4	31.5	-	143	40.0	7.29	142	55	0	-	0.327	3.90
5	34	-	-	-	-	142	47	0	-	0.206	3.25
6	36	-	-	-	-	142	47	0	-	0.309	3.50
7	38	-	-	-	-	142	43	0	-	0.248	2.40
8	41	-	133	42.0	7.30	142	39	0	-	0.218	2.15
9	51	-	-	-	-	142	33	0	-	0.182	1.50
10	56	-	140	38.0	7.31	142	33	2.86	-	0.703	2.50
11	61	-	-	-	-	142	33	4.39	-	-	1.10

DOG 26 (18.3kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	102	20.0	7.57	129	91	0.60	-	0.377	4.75
2	3.5	BR 1	-	-	-	111	150	0.92	-	0.350	4.75
3	10	CONTROL	79	22.0	7.53	129	83	1.16	-	0.311	4.35
4	12.5	BR 2	-	-	-	113	144	3.21	15	0.246	4.75
5	19	CONTROL	78	27.0	7.50	129	85	2.50	16	0.377	4.90
6	33	CONTROL	124	29.0	7.43	129	91	2.24	-	0.230	4.35
	34	LONG CHEMOTEST (20 minutes)									
7	35	LCR	43.5	44.0	7.22	129	93	3.84	15	0.311	5.20
8	37	LCR	47	30.0	7.31	129	89	2.14	-	0.344	4.25
9	39	LCR	42.5	35.0	7.28	124	131	2.15	-	0.235	4.35
10	41	LCR	47	38.0	7.26	124	170	2.13	-	0.279	4.80
11	43	LCR	47	36.0	7.22	118	156	2.29	-	0.251	4.60
12	46	LCR	47	35.0	7.21	109	143	2.29	-	0.339	4.40
13	51	LCR	47	30.0	7.21	96	96	2.87	26	0.301	5.05
14	56	-	205	23.0	7.34	96	81	4.06	12	0.322	4.85
15	61	-	215	26.0	7.31	104	56	0.48	-	0.164	1.40
16	66	-	175	26.5	7.27	129	69	0.78	-	0.333	1.95
17	71	-	-	-	-	129	89	1.13	-	0.311	3.00

DOG 26 (cont'd

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	81	-	-	-	-	129	78	1.17	-	0.301	2.45
19	91	-	-	-	-	129	85	1.37	-	0.322	2.90
	94	HYOSCINE METHYL BROMIDE (10mg/kg) + HEXAMETHONIUM BROMIDE (10mg/kg)									
20	112	CONTROL	165	26.0	7.34	129	80	0	-	0.306	2.80
	113	ACTH (25μg/min for 1 minute)									
21	114	ACTH	-	-	-	129	72	0	-	0.579	3.75
22	116	-	-	-	-	129	76	0	-	0.601	4.50
23	118	-	-	-	-	129	80	0	-	0.443	4.40
24	120	-	-	-	-	129	80	0	-	0.454	4.30
25	122	-	-	-	-	129	80	0	-	0.530	4.05
26	126	-	-	-	-	129	78	0	-	0.623	4.00
27	131	-	175	27.0	7.32	129	78	0	-	0.503	4.00

DOG 27 (25.0kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	128	44.5	7.33	129	118	9.20	10	0.312	3.75
2	3.5	BR 1	-	-	-	100	134	9.05	10	0.332	3.35
3	10	CONTROL	121	41.5	7.27	129	70	10.84	20	0.176	2.90
4	12.5	BR 2	-	-	-	100	94	9.74	8	0.228	2.95
5	21	CONTROL	99	42.5	7.32	129	54	9.37	12	0.508	4.20
	22	LONG CHEMOTEST (20 minutes)									
6	23	LCR	42.5	58.0	7.18	129	82	12.05	9	0.564	4.70
7	25	LCR	41	55.0	7.14	129	66	6.48	10	0.272	3.50
8	27	LCR	46.5	52.0	7.15	127	-	8.19	10	0.352	3.45
9	29	LCR	58.5	56.0	7.17	127	130	23.28	8	0.348	4.45
10	31	LCR	42.5	55.0	7.12	127	82	13.28	11	0.256	3.85
11	36	LCR	45.5	52.0	7.12	127	86	20.53	18	0.652	4.10
12	41	LCR	42.5	55.0	7.07	127	66	11.20	10	0.468	3.75
13	46	-	114	42.0	7.24	127	50	13.58	13	0.364	1.75
14	51	-	105	40.0	7.24	127	36	13.60	17	0.464	1.35
15	56	-	-	-	-	127	48	13.81	9	0.468	1.90
16	61	-	-	-	-	127	54	9.43	7	0.680	2.50
17	66	-	-	-	-	127	24	18.27	13	0.660	2.00

DOG 27 (cont'd)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	71	-	111	39.5	7.24	127	24	9.73	11	0.488	2.00
19	76	-	-	-	-	127	44	22.55	10	0.776	2.60
20	81	-	119	37.0	7.24	127	24	12.10	11	0.272	2.10

DOG 28 (8.5kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$	FLOW (ml/min)
1	1	CONTROL	134	32.0	7.32	100	57	1.34	-	1.141	2.60
2	3.5	BR 1	-	-	-	80	93	1.89	-	1.859	3.25
3	10	CONTROL	140	30.5	7.10	100	36	1.52	-	1.859	1.50
4	12.5	BR 2	-	-	-	82	83	1.49	-	1.965	2.30
	19						LONG CHEMOTEST (20 minutes)				
5	20	LCR	23.5	58.0	7.04	100	105	0.90	-	1.800	3.25
6	22	LCR	41.5	46.0	7.17	100	102	0.98	-	1.235	3.65
7	24	LCR	47	46.0	7.14	100	95	0.98	-	2.059	4.00
8	26	LCR	48.5	43.0	7.14	100	93	2.80	-	1.388	4.20
9	28	LCR	50.5	43.0	7.17	100	88	3.08	-	2.024	4.25
10	31	LCR	51.5	46.0	7.08	100	71	3.44	-	1.906	4.70
11	36	LCR	39	51.0	7.00	100	45	3.92	-	0.824	4.25
12	41	-	67	44.0	7.08	100	26	-	-	1.106	3.70
13	46	-	123	38.0	7.16	100	36	3.99	-	1.094	4.10
14	51	-	134	39.5	7.13	100	74	4.27	-	0.894	4.15
15	56	-	-	-	-	100	40	-	-	1.518	2.25
16	61	-	128	41.5	7.24	100	36	-	-	2.035	1.75
17	71	-	-	-	-	100	48	-	-	2.176	1.90
18	81	-	-	-	-	100	43	-	-	2.047	1.80

DOG 29 (8.1kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$	FLOW (ml/min)
1	1	CONTROL	172	44.0	7.25	126	75	1.57	19	0.235	1.05
2	3.5	BR 1	-	-	-	98	92	1.57	19	0.259	1.35
3	11	CONTROL	188	39.0	7.32	126	83	1.26	-	1.148	1.05
4	13.5	BR 2	-	-	-	98	92	1.26	-	1.012	1.10
5	19	CONTROL	175	36.0	7.31	126	85	0.91	-	1.321	1.15
	20	LONG CHEMOTEST (20 minutes)									
6	21.5	LCR	39.5	64.0	7.08	126	92	0.85	-	1.185	1.17
7	24.5	LCR	49	43.0	7.21	121	96	1.11	-	1.556	1.53
8	27.5	LCR	42.5	41.0	7.18	119	110	2.43	20	1.827	3.17
9	30.5	LCR	41	44.5	7.11	110	77	4.96	21	1.235	3.00
10	33.5	LCR	16.5	56.0	6.95	83	35	10.98	21	0.703	0.93
	40	DOG DEAD									

DOG 30 (14.6kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$	FLOW (ml/min)
1	1	CONTROL	94	30.0	7.41	121	108	6.16	41	-	3.80
2	3.5	BR 1	-	-	-	90	173	6.10	40	-	4.10
3	10	CONTROL	145	31.0	7.34	120	91	3.49	40	-	3.10
4	12.5	BR 2	-	-	-	89	164	2.95	36	-	4.90
5	21	CONTROL	165	29.5	7.34	125	107	7.05	70	-	3.45
	22	LONG CHEMOTEST (20 minutes)									
6	23	LCR	41	54.0	7.11	125	195	4.04	50	-	6.10
7	25	LCR	28.5	51.5	7.10	125	172	6.85	50	-	5.25
8	27	LCR	43.5	43.0	7.19	121	129	5.21	50	-	4.10
9	29	LCR	42	40.0	7.17	114	112	29.32	82	-	3.90
10	31	LCR	-	-	-	114	116	11.64	65	-	3.85
11	33	LCR	41.5	42.0	7.16	110	115	6.51	61	-	3.50
12	35	LCR	43	42.0	7.15	107	105	9.73	62	-	2.95
13	37	LCR	43	44.0	7.13	102	84	6.64	62	-	2.35
14	39	LCR	41.5	41.5	7.10	93	76	6.30	66	-	3.00
15	41	LCR	41.5	43.5	7.07	93	84	8.15	60	-	3.80
16	46	-	185	32.0	7.22	102	99	3.43	25	-	2.65
17	51	-	-	-	-	112	105	-	-	-	1.75

DOG 30 (cont'd)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
18	56	-	-	-	-	112	96	3.15	25	-	1.70
19	61	-	215	30.0	7.25	109	86	7.53	71	-	1.45
	80	CYCLOHEXIMIDE (50mg/kg)									
20	104.5	CONTROL	176	27.0	7.27	120	84	3.84	50	-	0.80

DOG 31 (9.1kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	195	33.0	7.30	121	66	3.30	27	-	1.80
2	3.5	BR 1	-	-	-	93	93	3.30	27	-	2.00
3	10	CONTROL	190	31.0	7.32	134	69	3.96	22	-	1.50
4	12.5	BR 2	-	-	-	96	111	3.74	40	-	1.75
5	19	CONTROL	190	39.0	7.36	134	71	4.40	29	-	1.50
	20	LONG CHEMOTEST (10 minutes)									
6	21	LCR	49.5	48.0	7.18	133	76	3.52	27	-	1.75
7	23	LCR	43	44.0	7.22	111	106	3.74	27	-	2.00
8	25	LCR	41	40.0	7.20	99	135	5.71	26	-	2.15
9	27	LCR	40	40.0	7.19	106	149	13.08	25	-	2.25
10	29	LCR	50	34.0	7.25	110	146	10.99	26	-	1.50
11	31	-	185	30.0	7.36	133	80	6.59	26	-	1.50
12	33	-	-	-	-	133	76	5.50	25	-	1.40
13	37	-	-	-	-	133	51	4.95	25	-	1.50
14	41	-	200	40.5	7.30	133	83	5.71	24	-	1.90
15	46	-	-	-	-	133	65	5.28	26	-	1.45
16	51	-	220	40.5	7.34	133	65	5.06	26	-	1.60
	60	CYCLOHEXIMIDE (50mg/kg)									

DOG 31 (cont'd)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
17	81	CONTROL	210	34.0	7.41	140	73	1.10	-	-	0.85
18	83.5	BR 3	-	-	-	112	89	1.32	-	-	0.95
19	90	CONTROL	-	-	-	142	90	2.20	-	-	0.75
20	92.5	BR 4	-	-	-	115	130	1.32	-	-	1.60
21	99	CONTROL	215	41.5	7.33	142	120	1.32	-	-	1.20
	100	LONG CHEMOTEST (10 minutes)									
22	101	LCR	9.5	105.0	6.92	139	158	1.10	-	-	1.30
23	103	LCR	42	48.0	7.20	135	156	1.10	-	-	1.40
24	105	LCR	43	49.0	7.20	135	151	1.43	-	-	1.18
25	107	LCR	44	48.0	7.21	135	146	1.98	-	-	1.10
26	109	LCR	43.5	42.0	7.21	135	132	1.98	-	-	1.00
27	111	-	200	25.0	7.42	135	124	2.97	-	-	1.20
28	113	-	220	23.0	7.44	135	87	2.64	-	-	0.85
29	118	-	-	-	-	135	82	1.76	-	-	0.50
30	121.5	-	-	-	-	135	90	2.20	-	-	0.73
31	126.5	-	-	-	-	139	81	2.42	-	-	0.60
32	131.5	-	238	21.0	7.49	140	72	2.20	-	-	0.53

DOG 32 (19.4kg M)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	1	CONTROL	215	35.0	7.37	123	114	2.72	16	-	0.85
2	3.5	BR 1	-	-	-	90	169	5.30	66	-	2.05
3	13	CONTROL	195	33.0	7.32	123	85	0.78	18	-	0.80
	15	LONG CHEMOTEST (20 minutes)									
4	16.5	LCR	47.5	39.5	7.19	123	117	8.25	56	-	2.17
5	19.5	LCR	37	37.0	7.20	121	102	12.37	39	-	2.92
6	22.5	LCR	36	35.0	7.17	118	125	17.22	30	-	3.50
7	25	LCR	36	33.0	7.18	118	130	21.29	38	-	4.00
8	27	LCR	36.5	35.0	7.22	118	123	5.21	22	-	4.05
9	29	LCR	36.5	33.0	7.19	118	124	2.94	19	-	3.85
10	31	LCR	41.5	30.0	7.20	118	124	3.09	18	-	3.10
11	36	-	200	27.0	7.36	118	115	3.20	15	-	3.30
12	41	-	-	-	-	120	88	1.55	15	-	0.90
13	46.5	-	196	28.0	7.32	119	76	1.75	22	-	1.13
14	51	-	-	-	-	119	72	1.55	16	-	2.75
15	61	-	-	-	-	123	88	3.40	18	-	3.50
	69	CYCLOHEXIMIDE (50mg/kg)									
16	111	CONTROL	206	29.0	7.25	121	121	1.80	25	-	1.85

DOG 32 (cont'd)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
17	113.5	BR 2	-	-	-	107	140	2.06	22	-	1.85
18	119	CONTROL	-	-	-	123	110	1.19	22	-	1.05
19	121.5	BR 3	-	-	-	103	135	3.45	25	-	0.65
20	126	CONTROL	175	28.0	7.25	122	96	1.29	25	-	1.60
	127					LONG CHEMOTEST (10 minutes)					
21	128	LCR	62.5	48.0	7.19	123	166	2.27	23	-	1.90
22	130	LCR	2.5	66.0	6.89	124	178	9.23	32	-	1.70
23	132	LCR	-	-	-	124	178	1.55	18	-	1.60
24	134	LCR	32	37.0	7.13	124	185	1.03	18	-	1.45
25	136	LCR	34.5	35.0	7.13	124	174	1.80	26	-	1.25
26	138	-	120	32.5	7.25	123	148	1.65	18	-	1.20
27	140.5	-	200	31.0	7.24	123	128	1.44	18	-	0.57
28	146.5	-	-	-	-	123	72	0.83	16	-	0.77
29	151.5	-	190	26.0	7.18	123	72	0.93	16	-	1.07
30	156.5	-	-	-	-	123	60	1.03	16	-	0.70
31	161.5	-	-	-	-	120	55	1.19	16	-	0.97

DOG 33 (17.5kg F)

NO.	TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP	SBP	ADRENAL OUTPUT			
			pO_2	pCO_2	pH	(mmHg)	(mmHg)	C.A. ($ng.kg^{-1}min^{-1}$)	NA%	HYDROCORTISONE ($\mu g.kg^{-1}min^{-1}$)	FLOW (ml/min)
1	0.63	CONTROL	96	34.0	7.34	102	85	4.06	26	1.623	7.44
2	3.13	BR 1	-	-	-	76	135	4.42	20	1.240	7.84
3	8.63	CONTROL	99.5	36.0	7.35	102	79	3.43	16	1.189	6.08
4	10.63	BR 2	-	-	-	78	140	4.53	21	1.069	7.04
5	13.63	CONTROL	101	30.0	7.36	102	71	2.78	18	1.103	4.80
	15					LONG CHEMOTEST (20 minutes)					
6	16.63	LCR	36	52.0	7.12	102	117	8.43	14	1.006	7.52
7	18.63	LCR	40	37.0	7.24	102	106	5.22	12	1.017	5.92
8	20.63	LCR	45	32.0	7.27	102	98	3.71	10	0.977	4.80
9	22.75	LCR	44	31.0	7.28	102	85	4.55	11	0.823	4.27
10	24.75	LCR	46	30.0	7.27	102	75	5.53	11	0.989	3.60
11	27	LCR	47.5	31.5	7.24	102	75	3.02	10	0.880	3.80
12	31	LCR	46.5	30.0	7.21	102	79	3.14	13	0.834	4.25
13	36		121	24.5	7.30	104	56	3.25	10	0.880	3.35
14	41		148	28.5	7.30	104	85	4.37	8	1.137	6.00
15	49		152	30.0	7.30	104	58	3.91	9	1.531	4.90
16	56		-	-	-	104	77	4.35	4	1.091	5.50
17	61		150	30.0	7.30	104	65	7.75	7	1.457	5.10
18	72.25		154	30.0	7.31	104	60	6.40	12	2.000	5.40

DOG 34 (10.3kg F)

TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	HLPP (mmHg)
		pO_2	pCO_2	pH			
0	CONTROL	-	-	-	155	57	84
1	BR 1	-	-	-	110	63	112
5	CONTROL	185	27	-	155	53	78
6.5	CR 1	42.5	39	-	155	53	110
11	CONTROL	-	-	-	155	47	72
12	BR 2	-	-	-	110	53	98
16	CONTROL	185	27	-	155	50	70
17.5	CR 2	40	39	-	155	50	96
20	HEXAMETHONIUM (10mg/kg)						
30	CONTROL	-	-	-	155	50	56
31	BR 3	-	-	-	110	57	58
35	CONTROL	190	28	-	155	57	58
36.5	CR 3	40	45	-	155	67	72
41	CONTROL	-	-	-	155	60	64
42	BR 4	-	-	-	110	67	66
46	CONTROL	124	29	-	155	57	60
47.5	CR 4	37.5	42	-	155	67	72

DOG 34 (Contd.)

TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	HLPP (mmHg)
		pO_2	pCO_2	pH			
51		HIND LIMB PERFUSION INCREASED					
55	CONTROL	-	-	-	155	83	90
56	BR 5	-	-	-	110	97	100
60	CONTROL	115	35	-	155	73	80
61.5	CR 5	35.5	47	-	155	103	122
68	CONTROL	-	-	-	155	73	76
69	BR 6	-	-	-	110	90	96
73		GUANETHIDINE (2mg/kg)					
81	CONTROL	128	33	-	155	97	94
82.5	CR 6	36	43	-	155	133	144
88	CONTROL	-	-	-	155	87	100
89	BR 7	-	-	-	110	87	100
93	CONTROL	130	34	-	155	90	98
94.5	CR 7	34	44	-	155	127	122
99	CONTROL	-	-	-	155	87	108
100	BR 8	-	-	-	110	87	108

DOG 34 (Contd.)

TIME (MINS)	TEST	CAROTID ARTERIAL BLOOD			CPP (mmHg)	SBP (mmHg)	HLPP (mmHg)
		pO_2	pCO_2	pH			
104		PHENTOLAMINE (10mg/kg)					
108	CONTROL	-	-	-	155	90	68
109	BR 9	-	+	-	110	90	68
113	CONTROL	122	31	-	155	83	66
114.5	CR 8	34	46	-	155	107	70
120	CONTROL	-	-	-	155	90	64
121	TYRAMINE ($100_{\mu}g/kg$)	-	-	-	155	97	64

Appendix 3

Tables of Results from Isolated Adrenal Gland Experiments

A. The Effect of Indomethacin on Catecholamine Release, Stimulated by Nicotine, from the Isolated Canine Adrenal Gland

Gland	Test	Time from start of perfusion (mins)	Catecholamine Release (ng/min)	% Noradrenaline	Stimulated Catecholamine Release (ng/min)
No. 1	CONTROL	60	724	34	-
	NICOTINE 10^{-5}M		1448	13	724
	CONTROL	120	470	18	-
	NICOTINE 10^{-4}M		1360	26	890
	CONTROL	165	418	7	-
	NICOTINE 10^{-3}M		776	7	358
	CONTROL	215	418	34	-
	NICOTINE 10^{-4}M		428	40	10
No. 2	CONTROL	180	266	2	-
	NICOTINE 5 x 10^{-5}M		1244	61	978
	CONTROL	230	296	17	-
	NICOTINE 5 x 10^{-5}M		852	80	556
	CONTROL	300	202	3	-
	NICOTINE 5 x 10^{-5}M		916	63	714
	INDOMETHACIN	330			
	LOCKES (1 µg/ml)				

A. (Contd.)

Gland	Test	Time from start of perfusion (mins)	Catecholamine Release (ng/min)	% Noradrenaline	Stimulated Catecholamine Release (ng/min)
No. 2 (Contd)	CONTROL	350	214	11	-
	NICOTINE 5 x 10^{-5}M		690	63	476
	CONTROL	390	198	3	-
	NICOTINE 5 x 10^{-5}M		568	52	370
No. 3	CONTROL	60	400	9	-
	NICOTINE 10^{-4}M		2964	21	2564
	CONTROL	105	348	37	-
	NICOTINE 10^{-4}M		934	46	586
	INDOMETHACIN	127			
	LOCKES (1 μg/ml)				
	CONTROL	150	406	7	-
	NICOTINE 10^{-4}M		556	30	150
	CONTROL	195	238	14	-
	NICOTINE 10^{-4}M		484	25	246

A. (Contd.)

Gland	Test	Time from start of perfusion (mins)	Catecholamine Release (ng/min)	% Noradrenaline	Stimulated Catecholamine Release (ng/min)
No. 4	CONTROL	80	104	20	-
	NICOTINE 2 x 10^{-5}M		260	19	156
	CONTROL	120	202	19	-
	NICOTINE 2 x 10^{-5}M		424	19	222
	INDOMETHACIN	142			
	LOCKES (1 µg/ml)				
	CONTROL	160	244	23	-
	NICOTINE 2 x 10^{-5}M		556	21	312
	CONTROL	200	316	2	-
	NICOTINE 2 x 10^{-5}M		650	18	334

B. The Effect of Prostaglandin E_2 on the Resting Catecholamine Release from the Isolated Canine Adrenal Gland

Gland	Test	Time from start of perfusion (mins)	Catecholamine Release (ng/min)	% Noradrenaline	Stimulated Catecholamine Release (ng/min)
No. 5	CONTROL	120	580	39	-
	PGE_2 0.2µg/ml		1804	12	1224
	CONTROL	160	510	9	-
	PGE_2 0.2µg/ml		1206	19	696
	CONTROL	195	464	2	-
	PGE_2 0.2µg/ml		950	2	486
	CONTROL	230	418	21	-
	PGE_2 0.6µg/ml		998	35	580
No. 6	CONTROL	180	412	8	-
	PGE_2 0.2µg/ml		742	9	330
	CONTROL	230	440	25	-
	PGE_2 0.2µg/ml		632	30	192
	CONTROL	450	290	20	-
	PGE_2 0.2µg/ml		650	19	360
	CONTROL	495	348	2	-
	PGE_2 0.1µg/ml		3118	14	2770

B. (Contd.)

Gland	Test	Time from start of perfusion (mins)	Catecholamine Release (ng/min)	% Noradrenaline	Stimulated Catecholamine Release (ng/min)
No. 6 (Contd)	CONTROL	540	534	2	-
	PGE_2 0.1 µg/ml		812	3	278
No. 7	CONTROL	75	1534	2	-
	NICOTINE 2×10^{-5} M		10072	35	8538
	CONTROL	115	892	22	-
	PGE_2 0.02 µg/ml		1670	17	778
	CONTROL	135	748	21	-
	PGE_2 0.1 µg/ml		1766	7	1018
	CONTROL	160	950	15	-
	PGE_2 0.2 µg/ml		1244	22	294
	CONTROL	185	882	21	-
	PGE_2 BLANK		870	20	-12
	CONTROL	240	838	24	-
	PGE_2 0.005 µg/ml		896	25	58
	CONTROL	246	954	33	-
	PGE_2 0.01 µg/ml		1100	31	146

B. (Contd.)

Gland	Test	Time from start of perfusion (mins)	Catecholamine Release (ng/min)	% Noradrenaline	Stimulated Catecholamine Release (ng/min)
No. 7 (Contd)	CONTROL	252	916	42	-
	PGE_2 0.02 µg/ml		994	27	78
	CONTROL	258	936	21	-
	PGE_2 0.04 µg/ml		1168	24	232
	CONTROL	264	752	16	-
	PGE_2 0.08 µg/ml		896	10	144
No. 8	CONTROL	150	460	41	-
	NICOTINE 5×10^{-5}M		7466	30	7006
	CONTROL	300	92	18	-
	PGE_2 0.2µg/ml		94	25	2
	CONTROL	405	138	39	-
	PGE_2 2 µg/ml		144	33	6
	CONTROL	450	166	45	-
	NICOTINE 5×10^{-5}M		166	55	0

C. The Effect of Hexamethonium on Catecholamine Release, Stimulated by Prostaglandin E_2, from the Isolated Canine Adrenal Gland

Gland	Test	Time from start of perfusion (mins)	Catecholamine Release (ng/min)	% Noradrenaline	Stimulated Catecholamine Release (ng/min)
No. 9	LOCKE'S SOLUTION CONTAINS HEXAMETHONIUM (3×10^{-5}M)				
	CONTROL	150	168	27	-
	NICOTINE 10^{-4}M		174	38	6
	CONTROL	195	226	4	-
	PGE_2 0.2 µg/ml		232	12	6
	CONTROL	240	208	6	-
	PGE_2 2 µg/ml		264	16	56
	CONTROL	285	220	2	-
	PGE_2 10µg/ml		226	3	6
	CONTROL	325	240	4	-
	NICOTINE 10^{-3}M		318	21	78
	CONTROL	340	244	2	-
	NICOTINE 10^{-2}M		600	5	356
No. 10	CONTROL	180	134	44	-
	NICOTINE 2×10^{-5}M		916	34	782
	CONTROL	225	214	26	-

C. (Contd.)

Gland	Test	Time from start of perfusion (mins)	Catecholamine Release (ng/min)	% Noradrenaline	Stimulated Catecholamine Release (ng/min)
No.10 (Contd)	PGE_2 0.2µg/ml		402	33	188
	CONTROL	260	194	13	-
	PGE_2 0.1µg/ml		344	2	150
	CONTROL	300	278	18	-
	PGE_2 0.02µg/ml		354	35	76
	CONTROL	340	266	7	-
	NICOTINE 2 x 10^{-5}M		574	36	308
	HEXAMETHONIUM	370			
	LOCKE'S (10^{-5}M)				
	CONTROL	410	256	8	-
	NICOTINE 2 x 10^{-5}M		388	26	132
	CONTROL	440	302	22	-
	PGE_2 0.1µg/ml		458	16	156
No. 11	CONTROL	60	1572	19	-
	NICOTINE 2 x 10^{-5}M		3698	14	2126
	CONTROL	120	1022	34	-

C. (Contd.)

Gland	Test	Time from start of perfusion (mins)	Catecholamine Release (ng/min)	% Noradrenaline	Stimulated Catecholamine Release (ng/min)
No.11 (Contd)	PGE_2 0.02 µg/ml		1476	26	454
	CONTROL	165	1322	31	-
	PGE_2 0.1 µg/ml		1766	26	444
	CONTROL	220	994	21	-
	PGE_2 0.2 µg/ml		1302	35	308
	HEXAMETHONIUM	265			
	LOCKE'S (10^{-5}M)				
	CONTROL	290	650	32	-
	PGE_2 0.1 µg/ml		800	37	150

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Publications

Effects of indomethacin on blood pressure, catecholamine release and adrenal blood flow in the anaesthetized, laparotomized dog

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A problem in studying adreno-medullary reflexes is the massive fall in blood pressure following laparotomy and handling of the viscera. Terragno *et al.* (1977) reported that laparotomy causes release of prostaglandins and that indomethacin, given after laparotomy, restores the blood pressure. We gave indomethacin (5 mg/kg) in divided doses to dogs before and after laparotomy. This enabled us to maintain a mean blood pressure around 100 mmHg for up to 3 h, whereas without indomethacin it was difficult to maintain a blood pressure of 70 mmHg.

In contrast to Feuerstein *et al.*'s (1979) results in the cat, indomethacin did not affect the amounts of adrenaline or noradrenaline released at rest or in response to chemoreceptor or barareceptor stimulation. Indomethacin did however reduce both the resting adrenal blood flow and the increase in blood flow during the reflex release of catecholamines.

The depression of the resting pressure flow curve (see Figure 1a) suggests the involvement of prostaglandins in maintaining adrenal blood flow. Figure 1b was derived using these curves to correct the flow changes accompanying the reflex rise in blood pressure. The results in Figure 1b after indomethacin imply a marked vasoconstriction associated with the

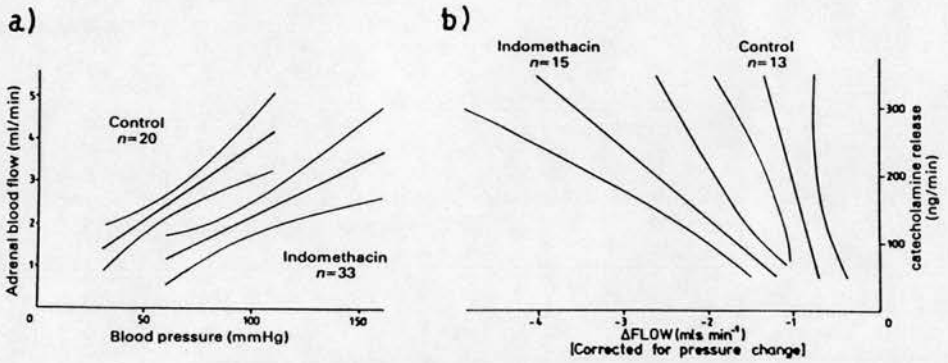

Figure 1 a) Resting pressure flow curves for left adrenal gland with and without indomethacin. b) Regression of change in adrenal flow (corrected for pressure from Figure 1a) on catecholamine release. All lines show 95% confidence limits.

release of catecholamines which is not seen without indomethacin. This suggests that prostaglandins are released with catecholamines and maintain adrenal blood flow by opposing their constricting action. This may also explain why platelet aggregation due to high concentrations of catecholamines does not occur in adrenal veins.

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THE REFLEX RELEASE OF ADRENALINE AND NORADRENALINE FROM THE ADRENAL GLANDS OF CATS AND DOGS

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SUMMARY

1. We have studied the release of noradrenaline and adrenaline from the adrenal glands of dogs and cats in response to the lowering of carotid sinus pressure (baroreceptor tests) and to the perfusion of the vascularly isolated carotid bifurcations with hypoxic blood (chemoreceptor tests).

2. In cats, the resting output of catecholamines had a ratio of noradrenaline to adrenaline of 1:1. The ratio in the incremental release during baroreceptor tests rose to 3:1, and during chemoreceptor tests it fell to 1:6.

3. In dogs, the ratio of noradrenaline to adrenaline at rest was 1:4. The ratio did not change over a wide range of outputs during baroreceptor tests, chemoreceptor tests and splanchnic nerve stimulation.

4. The release of catecholamines in response to baroreceptor tests in the cat was abolished by hexamethomium bromide at doses that did not diminish the response to chemoreceptor tests.

INTRODUCTION

In both the cat and the dog the adrenal glands release a mixture of adrenaline and noradrenaline into the bloodstream (Holtz *et al.* 1947; Bulbring & Burn, 1949). Many authors have argued for and against the independent control of the release of the two catecholamines. If one discounts work based on estimates of catecholamines in peripheral blood, where noradrenaline circulates from sympathetic adrenergic endings as well as from the adrenal glands, most of the evidence in favour of independent control comes from work on the cat (Von Euler & Folkow, 1953; Folkow & Von Euler, 1954; Düner, 1953), while most of the evidence against comes from work on the dog (Malmejac, 1964).

No direct comparison has been made between the two species, using similar techniques of stimulation, collection and assay. We therefore set out to apply to cats and dogs reflex stimuli that should, on the basis of previous work, preferentially release adrenaline or noradrenaline, and to assay both in the venous effluent of an adrenal gland.

Anichkov, Malyghina, Poskalenko & Ryzhenkov (1960) compared the effect of carotid occlusion with that of cyanide injection in cats. They found that the former produced a relatively greater vasoconstriction in a denervated hind limb, while the latter produced relatively greater contraction of a denervated nictitating membrane.

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These results suggest that the baroreceptor reflex may preferentially control noradrenaline release, and the chemoreceptor reflex preferentially control adrenaline release. We have therefore used baroreceptor and chemoreceptor stimuli to test the adrenal gland for independent release of adrenaline and noradrenaline.

A preliminary account of part of this work has been published (Critchley, Ungar & Welburn, 1973).

METHODS

Anaesthesia. Dogs were anaesthetized with an I.V. injection of chloralose (55 mg/kg) and urethane (550 mg/kg) or of sodium pentobarbitone (30 mg/kg) (see Discussion). Cats were anaesthetized with an I.P. injection of sodium pentobarbitone (40 mg/kg). In both species anaesthesia was maintained by continuous I.V. infusion of the anaesthetic agent at a rate of about one tenth of the initial dose per hour, adjusted so as just to suppress the paw withdrawal reflex.

Respiration, acid base balance and temperature control. The trachea was cannulated and connected to a Starling 'Ideal' pump. The lungs were ventilated with a metered oxygen-nitrogen mixture so as to hold P_{a,CO_2} at 5 kPa in dogs and at 4 kPa in cats, and P_{a,O_2} above 20 kPa in both species, measured from frequent arterial blood samples on a Radiometer BMS 3 analyser. A molar solution of sodium bicarbonate was injected after each sample to hold the arterial plasma pH at 7·4. Body temperature was held near to 37 °C by a heating pad linked to a rectal thermistor probe.

Carotid perfusion. Both common carotid arteries were cannulated both ways and blood from one of them was perfused into both towards the head, by a Watson Marlow MHRE pump. Both superior thyroid, internal carotid and external carotid arteries were ligated, and any other branches between the point of cannulation and the origins of the lingual arteries. Only the lingual arteries were left open to maintain an adequate flow through the system and thus allow changes in blood composition to affect the carotid bodies rapidly.

A pressure transducer was connected to the perfusion circuit. The signal was passed through a servo amplifier to the perfusion pump so that perfusion pressure could be set and held constant.

Stimulation of reflexes. Tests were performed, and the method evaluated as described by Henderson & Ungar (1978). Baroreceptor tests consisted of a lowering of carotid perfusion pressure from a constant resting level, while the P_{a,O_2} of the perfusing blood was held high. Chemoreceptor tests consisted of a lowering of the P_{a,O_2} of the perfusing blood, at constant perfusion pressure, while infusing into it a 1 M solution of sodium dithionite at a rate of about 150 mg/min (Critchley & Ungar, 1975). The duration of each test was 60 s in dogs and 120 s in cats.

The systemic arterial blood gas tension did not change whilst sodium dithionite was infused into the carotid circuit. In two dogs the application of lignocaine to both carotid sinus nerves completely abolished the vascular and respiratory responses to baroreceptor and chemoreceptor tests.

Both vagosympathetic trunks were cut in the neck in order to abolish secondary reflexes from thoracic receptors.

Collection of adrenal venous blood. In the dog, the left adrenolumbar vein was cannulated towards the gland, and the venous outflow collected into refrigerated heparinized tubes after ligation of the adrenal vein.

In the cat, cannulation of the adrenolumbar vein creates too great a back pressure on the gland. We therefore made a closed sac of the main vein into which the left adrenal gland drained: either the left renal vein or the inferior vena cava. The sac was bypassed by a silicone rubber tube. Adrenal venous blood was collected from the sac by a double-lumen cannula, the dead space of the sac being washed through by perfusing 10% sucrose solution at 4 ml min^{-1}.

Estimation of catecholamines. Adrenal venous blood was collected in centrifuge tubes containing measured volumes of 19% sucrose solution with EDTA 5 g/l and immediately centrifuged at 4 °C. The use of sucrose instead of salt was found to reduce the centrifugation time. The volumes of supernatant and of packed cells were recorded.

The samples were loaded onto Amberlite CG120 columns (mesh 100–200, length 20 mm,

diameter 2·5 mm). They were rinsed with 20 ml EDTA solution, 1 g/l, 2·5 ml phosphate buffer pH 6·5 and finally with 5 ml water. They were eluted with 4 ml M-hydrochloric acid and stored at 0 °C.

Trihydroxyindole derivatives were prepared by the method of Vendsalü (1960) with minor modifications (Critchley, 1976). This method gave an index of discrimination between adrenaline and noradrenaline of 8. Replicate estimates of plasma containing 5 nmol catecholamine/l gave a standard deviation of 0·5 nmol/l. The recovery of standards in plasma was 90%.

Analysis of results. The statistical significance of results was assessed by the paired *t* test.

Drugs. Chloralose and urethane (B.D.H. chemicals); sodium pentobarbitone (Abbott) and hexamethonium bromide (Koch Light).

RESULTS

Resting levels. The mean outputs of noradrenaline and adrenaline from the left adrenal glands of five dogs under chloralose (eighty-three samples) and seven cats (thirty samples) at rest are shown in Table 1. In the dog, noradrenaline and adrenaline were released with a ratio of about 1:4, and a mean total output of 77 pmol min^{-1} kg body wt^{-1}. In the cat, the ratio was 1:1 with a mean total output of 35 pmol min^{-1} kg body wt^{-1}. In a further three dogs under pentobarbitone anaesthesia the ratio was again 1:4, with a mean total output of 48 ± 8 pmol min^{-1} kg body wt^{-1}.

Baroreceptor and chemoreceptor tests. The results are shown in Table 1. Baroreceptor and chemoreceptor tests in both species gave two to threefold increases in catecholamine output. In the dog, there was no change in the ratio of noradrenaline to adrenaline during either of the reflex responses. In the cat, on the other hand, the ratio of noradrenaline to adrenaline rose from 1:1 to 3:1 in the increment over control output during baroreceptor tests, and fell from 1:1 to 1:6 in the increment over control output during chemoreceptor tests. These changes are statistically significant ($P < 0{\cdot}01$).

The effect of magnitude of response on the ratio of catecholamines released. In both dogs and cats we investigated the effect of varying the intensity of chemoreceptor and baroreceptor tests on the ratio of noradrenaline to adrenaline released. The results are shown in Fig. 1 in the form of a regression analysis of ratio on total release. Over a more than fivefold range of rate of release in each group, the ratio in cats rose significantly above the resting ratio during baroreceptor tests, and fell significantly below it during chemoreceptor tests. In dogs the ratio remained fixed throughout the ranges of both stimuli.

Tests on dogs under pentobarbitone anaesthesia. In view of the possibility that the selective release of noradrenaline and adrenaline could be due to anaesthetic rather than species differences, we carried out baroreceptor and chemoreceptor tests on five dogs under pentobarbitone anaesthesia. In six baroreceptor tests, in which the carotid sinus pressure was lowered from 120 to 90 mmHg, the mean total catecholamine output rose from 48 ± 8 to 105 ± 15 pmol min^{-1} kg^{-1}. In six chemoreceptor tests the mean total catecholamine output rose from 48 ± 8 to 80 ± 41 pmol min^{-1} kg^{-1}. The ratio of noradrenaline:adrenaline at rest was $0{\cdot}25 \pm 0{\cdot}3$, during baroreceptor tests was $0{\cdot}22 \pm 0{\cdot}03$ and during chemoreceptor tests was $0{\cdot}20 \pm 0{\cdot}03$.

Thus qualitatively the reflex responses of dogs under chloralose and under pentobarbitone were similar. Quantitatively the adrenal medulla was more responsive to baroreceptor tests under pentobarbitone than under chloralose. The responses to

TABLE 1. Outputs of noradrenaline (NA) and adrenaline (A) from the left adrenal glands of dogs and cats, at rest and during baroreceptor and chemoreceptor tests. * indicates data significantly different from the corresponding controls ($P < 0{\cdot}01$)

	Carotid perfusate			Artenal pressure (mmHg)	Left adrenal venous effluent		
	P (mmHg)	P_{O_2} (kPa)	P_{CO_2} (kPa)		NA (pmol min^{-1} kg^{-1})	A (pmol min^{-1} kg^{-1})	NA %
				Dogs ($n = 5$)			
Control	142 ± 2	24 ± 3	5·4 ± 0·3	96 ± 13	14 ± 2	65 ± 5	19 ± 1
Baroreceptor test ($n = 36$)	84 ± 2	24 ± 3	5·4 ± 0·3	135	39	171	—
Increments	—	—	—	39 ± 14	25 ± 4*	106 ± 22*	19 ± 2
Control	142 ± 2	24 ± 3	5·4 ± 0·3	96 ± 13	14 ± 2	65 ± 5	19 ± 1
Chemoreceptor test ($n = 23$)	142 ± 2	4·4 ± 0·6	7·7 ± 0·7	179	27	138	—
Increments	—	—	—	83 ± 9	13 ± 2*	73 ± 17*	20 ± 3
				Cats ($n = 7$)			
Control	150 ± 2	28 ± 3	3·6 ± 0·1	81 ± 5	18 ± 3	17 ± 2	46 ± 4
Baroreceptor test ($n = 14$)	84 ± 4	28 ± 3	3·6 ± 0·1	140	37	26	—
Increments	—	—	—	59 ± 8	19 ± 4*	9 ± 2*	74 ± 6*
Control	149 ± 1	35 ± 6	3·9 ± 0·3	84 ± 5	18 ± 3	17 ± 2	46 ± 4
Chemoreceptor test ($n = 10$)	149 ± 1	5 ± 2	9·2 ± 1·5	164	48	89	—
Increments	—	—	—	83 ± 10	30 ± 17*	72 ± 3*	16 ± 5*

chemoreceptor tests, however, were substantially but variably inhibited by pentobarbitone. Except in one dog, lowering of the carotid perfusate P_{O_2} to between 4 and 5 kPa did not release catecholamines, although strong respiratory and vascular responses were obtained with stimuli of this intensity, but release was obtained in chemoreceptor tests where the P_{O_2} fell to 3 kPa.

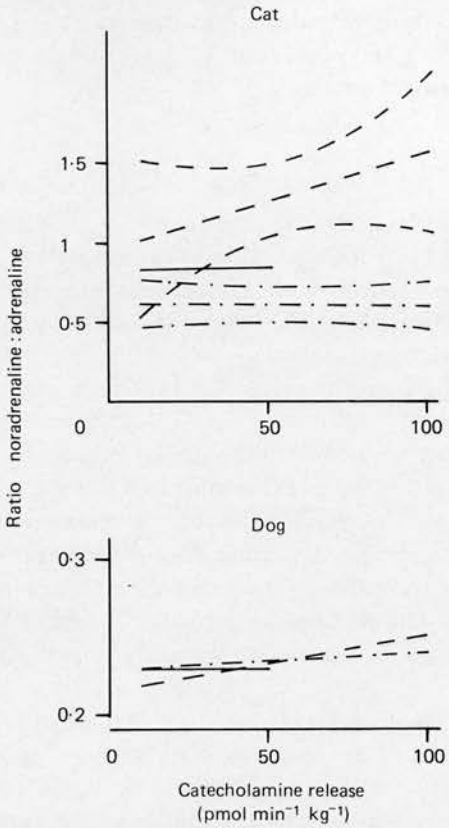

Fig. 1. The relationship between total catecholamine release and the ratio of noradrenaline to adrenaline. Regression lines of noradrenaline:adrenaline on total release. Continuous lines represent resting release, dashed lines release during baroreceptor tests, and dashed-dotted lines release during chemoreceptor tests. The upper graph represents results on five cats, with fifteen resting levels, nine chemoreceptor tests and twelve baroreceptor tests. The lower graph represents results in five dogs with thirty resting levels, fourteen chemoreceptor tests and fourteen baroreceptor tests. For each line the range of total release is greater than 5:1. The lines for chemoreceptor tests and baroreceptor tests in cats show 95% confidence limits. Note that the scale of the ordinate for dogs is 10 times that for cats.

The effect of hexamethonium bromide on baroreceptor and chemoreceptor tests. In three cats, we carried out baroreceptor and chemoreceptor tests before and after I.V. injection of hexamethonium bromide (2 mg/kg body wt). The mean resting output of catecholamines was 36 ± 4 pmol min^{-1} kg^{-1}. This rose by 38 ± 17 pmol min^{-1} kg^{-1} during baroreceptor tests and by 22 ± 11 pmol min^{-1} kg^{-1} during chemoreceptor tests. After administration of hexamethonium bromide the resting output

was 14 ± 3 pmol min^{-1} kg^{-1}. The output now rose by $0{\cdot}05 \pm 2$ pmol min^{-1} kg^{-1} during baroreceptor tests, and by 41 ± 21 pmol min^{-1} kg^{-1} during chemoreceptor tests. The response to chemoreceptor tests was thus undiminished by a dose of hexamethonium bromide that abolished the response to baroreceptor tests.

Electrical stimulation of the greater splanchnic nerve. In three dogs fifteen tests of electrical stimulation of the greater splanchnic nerve were performed. During stimulation, the mean output of noradrenaline rose by 224 ± 63 pmol min^{-1} kg^{-1}, and that of adrenaline by 712 ± 190 pmol min^{-1} kg^{-1}, giving a ratio not significantly different from that in the resting output.

DISCUSSION

Anaesthesia. The choice of anaesthetic agents was a major problem. We found that in cats under pentobarbitone we were able to obtain balanced adrenal responses to chemoreceptor and baroreceptor tests, from a low resting level. Cats under chloralose have high resting outputs with a high ratio of noradrenaline to adrenaline (Kaindl & Von Euler, 1951) and also show selective depression of baroreceptor responses in contrast to chemoreceptor responses (Neil, Redwood & Schweitzer, 1949).

In dogs on the other hand we obtained balanced responses under chloralose to stimuli of the same order of intensity as those required in cats under pentobarbitone. In dogs under pentobarbitone, the chemoreceptor response was strongly inhibited in relation to the baroreceptor response. By using stronger stimuli we were nevertheless able to exclude any shift in the ratio of noradrenaline to adrenaline in the adrenal effluent of dogs with either stimulus under pentobarbitone anaesthesia. The differences between dogs and cats in our experiments are not due to differences in anaesthesia.

Resting levels and size of responses. Our resting outputs of catecholamines in the dog are similar to those reported by previous workers who took similar precautions to avoid excessive blood loss or hypoxia (Rapela & Houssay, 1952; De Schaepdryver, 1959). Our resting levels in the cat are similar to those reported by Feurstein & Gutman (1971) for cats under pentobarbitone anaesthesia.

The size of our reflex responses is similar to that obtained by De Schaepdryver (1959) with carotid occlusion, but far smaller than those found by other workers using more massive stimuli such as haemorrhage and asphyxia (Rapela & Houssay, 1952). The responses to electrical stimulation show that our preparations are capable of maximal outputs of catecholamines far greater than their reflex responses to discrete sensory stimuli.

Selective release of noradrenaline and adrenaline. Our results in the cat provide direct confirmation for the conclusion of Anichkov *et al.* (1960) that the arterial baroreceptors selectively control noradrenaline output from the adrenal glands while the arterial chemoreceptors selectively control adrenaline output. Since we are dealing with concentrations of catecholamines in the adrenal venous effluent, there is no question of our results being confused by noradrenaline circulating from peripheral sympathetic endings.

In the dog we have found no evidence for selective control of the release of

noradrenaline or of adrenaline from the adrenal medulla. Our results are compatible with those of previous workers on the dog (Malmejac, 1964; De Schaepdryver, 1959; Wurtman, Casper, Pohorecky & Bartler, 1968) who failed to find evidence for selective release from the adrenal medulla in response to physiological stimuli.

Having studied the reflex release of catecholamines in substantially similar preparations in dogs and cats, we support the view that the controversy on selective release can be resolved by the species difference in the control of the adrenal medulla between dogs and cats. In the dog the ratio of noradrenaline to adrenaline does not deviate from about 1:4 over a wide range of outputs. In the cat, on the other hand, the resting output has a ratio of about 1:1, but when release is stimulated the ratio can swing at least between 1:6 and 3:1.

The effect of hexamethonium bromide. We found the release of catecholamines in response to baroreceptor stimulation in the cat to be abolished by hexamethonium bromide at a dose that did not diminish similar responses to chemoreceptor stimulation. This finding may be relevant to the observation of Douglas & Poisner (1965), in isolated cat adrenal glands, that noradrenaline is preferentially released by nicotinic agonist drugs and adrenaline by muscarnic agonists. It also matches the findings of Henderson & Ungar (1978) that reflex vasoconstriction in the hind limb of the dog in response to baroreceptor tests is selectively inhibited by hexamethonium bromide, and that to chemoreceptor tests by hyoscine methyl bromide. There thus appear to be parallel nicotinic and non-nicotinic pathways both to chromaffin cells and to sympathetic ganglia mediating baroreceptor and chemoreceptor reflexes respectively.

We are grateful for the expert technical assistance of Mr P. H. Whelpdale. P. Ellis is an M.R.C. Training Scholar.

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With 4 text-figures

THE ROLE OF THE PITUITARY–ADRENOCORTICAL AXIS IN REFLEX RESPONSES OF THE ADRENAL MEDULLA OF THE DOG

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SUMMARY

1. The release of catecholamines from the adrenal medulla, in response to carotid body hypoxia, may outlast the stimulus by more than 30 min.

2. After denervation of the adrenal gland the immediate release of catecholamines in response to carotid hypoxia is abolished, but the prolonged release remains.

3. The prolonged release of catecholamines is abolished by cycloheximide.

4. Both corticotrophin *in vivo* and hydrocortisone in the isolated perfused adrenal gland release adrenomedullary catecholamines.

5. It is concluded that a component of the response of the adrenal medulla to carotid body hypoxia is mediated by corticotrophin and corticosteroid release.

INTRODUCTION

In previous work from this laboratory we studied the response of the adrenal medulla to short hypoxic stimuli, localized to the carotid bifurcations, lasting 30–60 sec (Critchley, Ungar & Welburn, 1973; Critchley, 1976; Critchley, Ellis & Ungar, 1980). We always found that the release of catecholamines began and ended sharply with the beginning and end of the stimulus, as one would expect of a reflex response mediated by the autonomic nervous system. We observed, however, that if the stimulus was prolonged to 10 min, the release of catecholamines outlasted the stimulus by at least a further 30 min, although both the adrenal glands and the central nervous system remained perfused with well-oxygenated blood throughout the experiment.

Carotid chemoreceptor stimulation releases corticotrophin from the anterior lobe of the pituitary gland, and thus corticosteroids from the adrenal cortex (Anichkov, Malyghina, Poskalenko & Ryzenkov, 1960; Marotta, 1972). The conversion of noradrenaline to adrenaline in the mammalian chromaffin cell depends on the induction of the enzyme phenylethanolamine *N*-methyl transferase (PNMT) by the high concentration of corticosteroids that reaches the adrenal medulla through direct portal channels from the cortex (Wurtman, Pohorecky & Baliga, 1972). Little is known, however, of the influence of corticosteroids upon the release, as distinct from the synthesis, of medullary catecholamines.

We have now investigated the possibility that the sustained release of catecholamines in response to transient carotid hypoxia could be mediated by the pituitary–adrenocortical axis.

Preliminary reports of parts of this work have been published (Critchley & Ungar, 1974; Critchley, Henderson, Moffatt, Ungar, Waite & West, 1975).

METHODS

Anaesthesia, respiration, acid–base balance and temperature control

Dogs were anaesthetized with an I.V. injection of chloralose (55 mg/kg) and urethane (550 mg/kg). Anaesthesia was maintained by a continuous I.V. infusion of the anaesthetic mixture adjusted so as just to suppress the paw withdrawal reflex. The trachea was cannulated and connected to a Starling 'Ideal' pump. The lungs were ventilated with a metered oxygen–nitrogen mixture so as to hold P_{a,CO_2} at 5 kPa and P_{a,O_2} above 20 kPa, measured from frequent arterial blood samples on a Radiometer BMS3 analyser. A molar solution of sodium bicarbonate was injected when necessary to restore arterial plasma pH to 7·4. Body temperature was held near to 37 °C by a heating pad controlled from a rectal thermistor probe. Heparin (500 i.u./kg) was injected after the completion of surgery. Dextran was infused if necessary to prevent the mean systemic arterial pressure from falling below 75 mmHg.

Carotid perfusion

Both common carotid arteries were cannulated both ways, and blood from one was perfused into both arteries towards the head by a Watson Marlow MHRE pump. The superior thyroid, internal carotid and external carotid arteries and any other branches between the point of cannulation and the origins of the lingual arteries, were ligated. The lingual arteries were left open to maintain an adequate flow through the system and thus allow changes in the perfusing blood to affect the carotid bodies quickly, but were occluded to test the vascular isolation of the carotid bifurcations. A pressure transducer was connected to the perfusion circuit and linked through a servo amplifier to the perfusion pump so that perfusion pressure could be held constant, or varied at will.

Stimulation of reflexes

Chemoreceptor tests were performed and evaluated as described by Henderson and Ungar (1978). They consisted of a lowering of the P_{O_2} of the perfusing blood by infusing into it a solution of sodium dithionite (Critchley & Ungar, 1975). Carotid sinus pressure was held during chemoreceptor tests at 150 or 140 mmHg. Baroreceptor tests were performed by lowering the carotid perfusion pressure by 40 mmHg for 60 sec.

Both vagosympathetic trunks were divided in the neck to abolish secondary reflexes from thoracic receptors. In two dogs the application of lignocaine (2 % solution) to both carotid sinus nerves abolished the release of catecholamines, as well as the respiratory and cardiovascular responses to chemoreceptor and baroreceptor tests.

Collection of adrenal venous blood and estimation of catecholamines

The left adrenolumbar vein was cannulated towards the gland, and the adrenal vein ligated between the gland and the vena cava. The outflow was collected for timed periods in cooled tubes. The concentrations of adrenaline and noradrenaline in the plasma were estimated fluorometrically, after extraction on ion exchange columns, as described by Critchley *et al* (1980). By catecholamine concentration, throughout this paper, we mean the sum of the concentrations of adrenaline and noradrenaline, separately estimated. The fraction of adrenaline in the mixture did not vary in resting or evoked releases outside the range of 75–80 %. Replicate estimates of plasma containing 5 nM-catecholamine gave a standard deviation (S.D.) of 0·5 nM. The recovery of standards from plasma was 90 %.

Arterial concentrations of catecholamines during reflex responses did not exceed 2 × plasma blanks.

Denervation of the left adrenal gland

The left splanchnic nerve was identified and cut about 30 mm from the adrenal gland. The retroperitoneal connective tissue was dissected in an arc around the gland to interrupt additional sympathetic fibres, particularly around the arteries supplying the gland. The abolition of the

adrenal response to baroreceptor tests, while the pressor response remained, was taken as evidence of complete denervation.

Isolated adrenal glands

Dogs, used for unrelated acute experiments, were anaesthetized and heparinized as above. Each adrenolumbar vein was cannulated towards the gland, the adrenal vein tied between the gland and the vena cava, and the gland excised leaving its arterial orifices open. The gland was flushed through with Locke's solution (Na^+, 156 mM; K^+, 5·6 mM; Ca^{2+}, 4·3 mM; Cl^-, 164 mM; HCO_3^- 1·8 mM; glucose 5 mM) and suspended by its cannula from a Langendorff isolated heart perfusion apparatus, perfused with Locke's solution at 37 °C at a constant flow of 2 ml./min.

The effluent from the arterial orifices was collected for 30 sec periods in glass tubes in a Unicam AC60 autoanalyser, which delivered the reagents for catecholamine assay, without the preliminary extraction as for plasma.

Drugs

Chloralose, (BDH); corticotrophin, (Ciba); cycloheximide, (Sigma); heparin, (Evans); hydrocortisone, (Glaxo); urethane, (BDH).

RESULTS

Resting output of catecholamines in dogs with innervated adrenal glands

In twenty-two dogs the mean resting output of catecholamines from the left adrenal gland was 57 p-mole/min . kg, with a S.D. of 20. In those dogs which did not undergo irreversible procedures, such as denervation, the resting levels remained stable over the duration of the experiment; over a period of 90 min the S.D. of the output did not exceed 25% of the resting output.

Chemoreceptor tests in dogs with innervated adrenal glands

In each of six dogs a baroreceptor test, lasting 1 min, and 10 min later a chemoreceptor test, of 10 min duration, were performed. The mean output of catecholamines in the course of these tests is shown in the top panel of Fig. 1. In each test a rapid response was followed by a sustained output of catecholamines which outlasted the stimulus by 20–60 min. During these tests the mean systemic blood pressure rose from 73 ± 8 to 105 ± 20 mmHg.

Chemoreceptor tests in dogs with denervated adrenal glands

A secretory response which outlasts the stimulus that evoked it by more than a few minutes is more likely to be mediated by a humoral than by a neural mechanism. The crucial test of such a hypothesis is to divide the motor nerves supplying the effector organ.

In six dogs with denervated left adrenal glands, baroreceptor tests lasting 1 min were performed to confirm the total denervation of the gland. Ten minutes later chemoreceptor tests, lasting 20 min, were performed. The mean results are shown in the middle panel of Fig. 1. The output of catecholamines did not rise during the baroreceptor test, although the usual reflex rise in systemic blood pressure was seen. All the denervated glands responded to chemoreceptor tests with a rise in catecholamine output but, in contrast to the response of innervated glands, a rise was not seen during the first 5 min of a test. The time course of a single response is shown in Fig. 2.

Chemoreceptor tests in dogs with fully isolated carotid bifurcations

In three dogs the lingual arteries were cannulated, completing the vascular isolation of the carotid bifurcations, and blood was returned through a resistance to a femoral vein. Two of the dogs had denervated left adrenal glands. They gave no response in baroreceptor test nor in the early phase of chemoreceptor tests, but

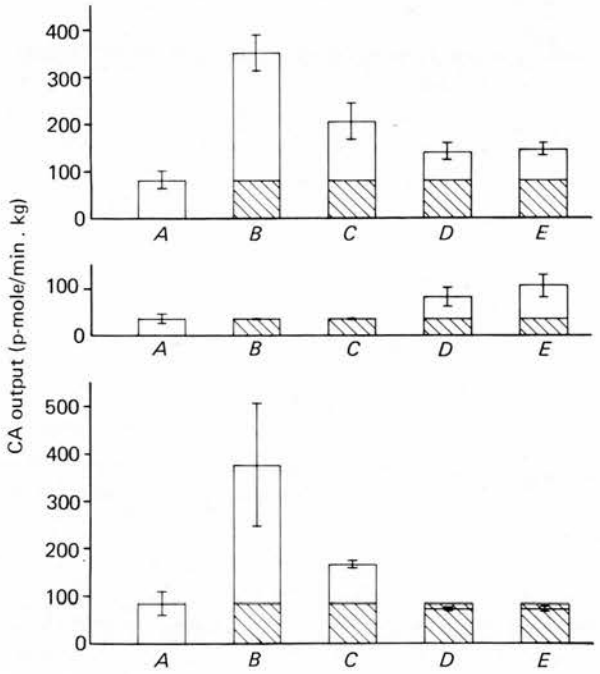

Fig. 1. Responses of dogs to baroreceptor and chemoreceptor tests. Mean responses of three groups of six dogs. The columns represent the output of catecholamines from the left adrenal gland (CA output). In each panel column *A* is the resting output, *B* the output during a baroreceptor test, *C* the output 5 min after the start of a chemoreceptor test, *D* the output 10 min after the end of a chemoreceptor test and *E* the output 20 min after the end of a chemoreceptor test. The vertical bars represent the S.E. of the mean output in *A* and of the mean increment above resting level (hatched) in *B–E*. The top panel shows the response of untreated dogs. In the chemoreceptor tests, the P_{O_2} of the carotid perfusate was reduced from 15 ± 1 to $5 \pm 0{\cdot}2$ kPa for 10 min. The middle panel shows the responses of dogs with denervated left adrenal glands. In the chemoreceptor tests, the P_{O_2} of the carotid perfusate was reduced from 18 ± 2 to $5 \pm 0{\cdot}2$ kPa for 20 min. The bottom panel shows the responses of dogs given cycloheximide 50 mg/kg, 20 min before the test. In the chemoreceptor tests, the P_{O_2} of the carotid perfusate was reduced from 16 ± 3 to $4 \pm 0{\cdot}3$ kPa.

catecholamine output rose to 3× and 4× the resting output 20 min after the end of the chemoreceptor stimulus. The third dog, whose adrenal innervation was intact, showed a 3× increase in output in a baroreceptor test, and 4× increases in output both during and 20 min after the chemoreceptor test.

The patterns of these responses are identical with those of dogs having open lingual arteries, and thus exclude the possibility that any part of the responses could be mediated by structures reached directly by lingual arterial blood.

The effect of cycloheximide on the responses of innervated glands to chemoreceptor and baroreceptor tests

Cycloheximide is a drug which inhibits the release of corticosteroids in response to corticotrophin (Garren, Ney & Davis, 1965). We performed tests before and after the I.V. injection of cycloheximide (50 mg/kg) in six dogs. The results are shown in the bottom panel of Fig. 1. The immediate release of catecholamines during both

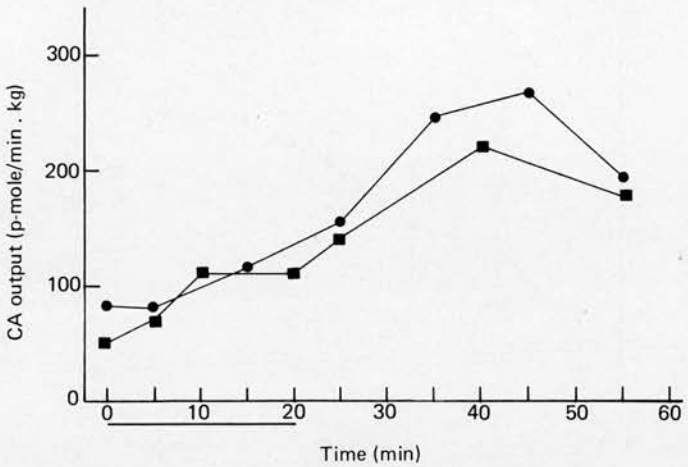

Fig. 2. The time course of the responses to chemoreceptor tests and to corticotrophin. Time course of the output of catecholamines (CA output) from the denervated left adrenal glands of two dogs. Circles indicate the response to a chemoreceptor test lasting 20 min and squares the response to an infusion of corticotrophin 25 μg over 20 min.

chemoreceptor and baroreceptor tests was not impaired by cycloheximide. In all six dogs, however, the sustained release in response to the chemoreceptor tests was abolished by cycloheximide.

These results support the idea of there being two components to the chemoreceptor response, of which the late component is abolished by cycloheximide.

The effect of exogenous corticotrophin on adrenomedullary catecholamine output in vivo

In six dogs corticotrophin (25 μg) was infused intravenously. The mean results are shown in Fig. 3 and the time course of a single response in Fig. 2. In two of these dogs regular baroreceptor tests were performed before and after the administration of corticotrophin. The release of catecholamines during baroreceptor stimulation was potentiated 3–4-fold at the peak of the response to corticotrophin.

Corticotrophin increased the output of catecholamines with a similar time course to that shown by prolonged chemoreceptor tests. At the peak of the action of corticotrophin the response to baroreceptor tests was potentiated.

In three dogs corticotrophin (25 μg) was infused 50 min after the injection of cycloheximide (50 mg/kg). The mean output of catecholamines rose from 21 ± 12 p-mole/min . kg by a mean of 13 ± 5 p-mole/min . kg. This can be compared with

the rise in dogs untreated with cycloheximide, from a mean of 86 ± 16 p-mole/min . kg by a mean of 216 ± 76 p-mole/min . kg.

The release of catecholamines by hydrocortisone from isolated perfused adrenal glands

Seven isolated adrenal glands were perfused. Hydrocortisone was added to each perfusate at concentrations of 30, 50 and 100 μg/ml. for 5 min periods. The effect on the catecholamine output into the effluent is shown in Fig 4 *A*. These concentrations

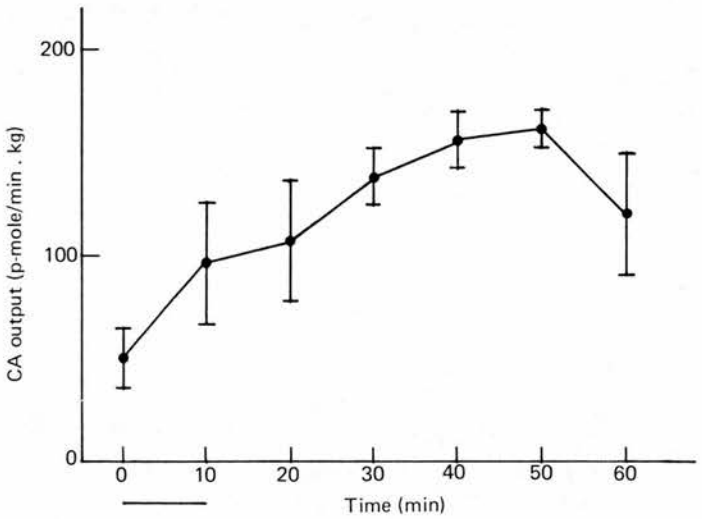

Fig. 3. The response of the adrenal medulla to corticotrophin. Mean output of catecholamines from the left adrenal glands of six dogs (CA output). The bar indicates the intravenous infusion of 25 μg corticotrophin. Vertical bars represent S.E.

of corticosteroid are within the physiological range for adrenal portal blood, if one assumes that the adrenal blood flow was of the order of 1 % of the cardiac output.

The time course of the response to the middle dose of hydrocortisone is shown in Fig. 4*B*. The time of onset of the release of catecholamines is similar to that of the late component of the response to chemoreceptor tests, and to that of the response to corticotrophin.

Three isolated adrenal glands were each perfused for 10 min with Locke's solution containing cycloheximide (1 mg/ml.), a concentration far higher than that circulating in the experiments *in vivo*. There was no change in the output of catecholamines.

DISCUSSION

We have previously reported (Critchley *et al.* 1980; Critchley, 1976) that in baroreceptor and chemoreceptor tests lasting 1 min the output of catecholamines from the left adrenal gland rose to between 2 × and 5 × the resting output. The output invariably returned to or to below the resting level within 2 min after baroreceptor tests, and within 5 min after chemoreceptor tests. The last figure is certainly an

overestimate, because of the time taken to wash hypoxic blood out of the perfusion system.

In the present experiments we used chemoreceptor tests lasting 10 or 20 min, and the response was always sustained for 20–60 min after the end of the stimulus. Although the hypoxic stimulus was milder than that used in the short tests, the peak

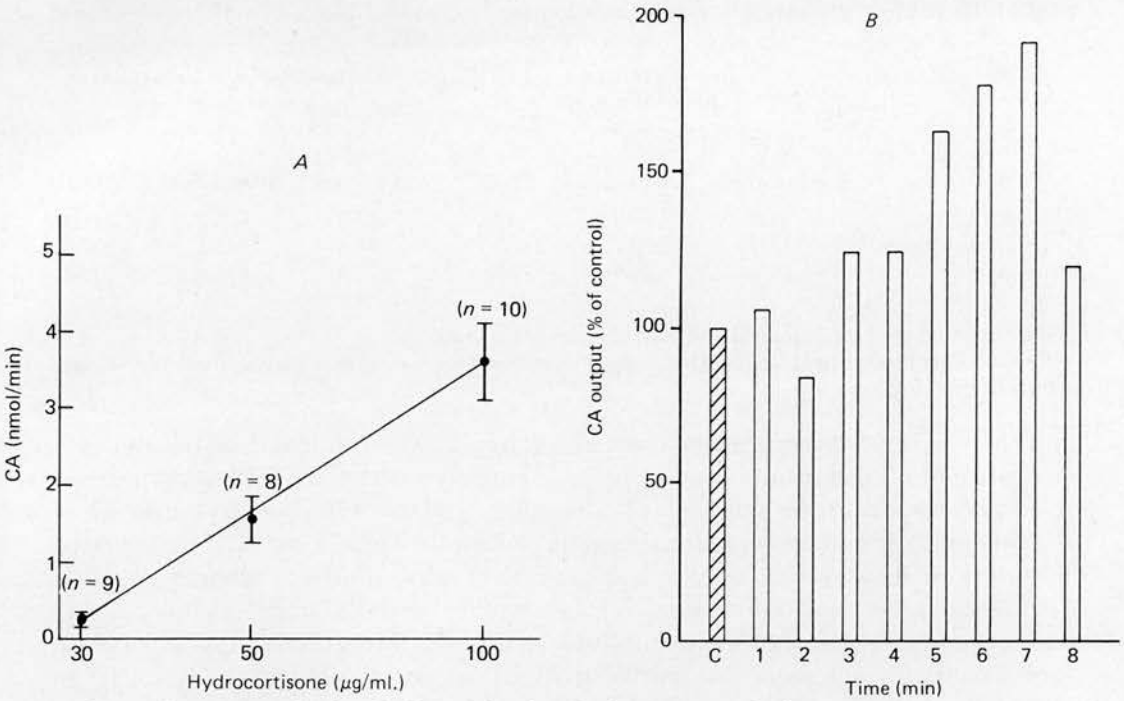

Fig. 4. Responses of isolated adrenal glands to hydrocortisone. *A*, the mean resting output of catecholamines (CA) from seven canine glands perfused with Locke's solution at 2 ml./min and 37 °C was 2·6 ± 1·0 n-mole/min. The graph shows the incremented outputs when the glands were exposed to three concentrations of hydrocortisone in the perfusate. The vertical bars indicate standard deviations and the numbers of tests are shown in brackets. *B*, the mean time course of the normalized output of catecholamines (CA output) in response to the middle concentration of hydrocortisone shown in *A*, added to the perfusate for 5 min as indicated by the horizontal bar.

output of catecholamines was greater. We were able to separate the response into a fast and a slow phase. The fast phase, together with the baroreceptor response, was abolished by denervation of the adrenal gland. The slow phase was abolished by cycloheximide, and corresponded in its time course with the response to exogenous corticotrophin.

We conclude from our results that there are two components to the response of the adrenal medulla to arterial chemoreceptor stimulation. The rapid component requires an intact nerve supply to the adrenal gland, but is independent of adrenocortical function. The delayed component, on the other hand, requires an intact pituitary–adrenocortical axis, but is independent of the motor nerves to the gland.

We were surprised to find no previous investigation of the direct effect of adrenocortical function on the release of adrenal catecholamines, although their action on the synthesis of adrenaline has been studied in detail. Wurtman, Casper, Pohorecky & Bartter (1968) studied the release of adrenomedullary catecholamines in dogs in response to insulin-hypoglycaemia. They compared the responses of normal dogs

TABLE 1. Insulin hypoglycaemia in normal dogs, hypophysectomized dogs, and hypophysectomized dogs treated with corticotrophin

	Resting output (p-mole/min . kg)			Peak response to insulin (p-mole/min . kg)		
	Noradrenaline	Adrenaline	Total	Noradrenaline	Adrenaline	Total
Normal	8·6	40·5	49·1	37·8	172·3	210·1
Hypophysectomized	14·0	22·7	36·7	51·3	84·2	135·5
Hypophysectomized + corticotrophin	16·7	38·9	55·6	174·4	401·8	576·2

Data recalculated from the results of Wurtman *et al.* (1968).

Resting output and peak stimulated output of adrenaline and noradrenaline from the adrenal glands of five dogs.

with those of hypophysectomized dogs, and of hypophysectomized dogs treated with corticotrophin. Their results were interpreted entirely with regard to changes induced by corticosteroids in the synthesis of adrenaline, and are expressed as outputs and percentages of adrenaline in adrenal venous plasma. In Table 1 we have recalculated their data to show resting and evoked outputs of adrenaline and noradrenaline. It is evident that corticotrophin increased the absolute output of noradrenaline, as well as that of adrenaline, in the resting state and particularly during insulin hypoglycaemia. Their results do not seem compatible with an action of corticosteroids solely on adrenaline synthesis. They are more simply explained by an action on the release of catecholamines, and suggest both direct release by steroids and the potentiation of the effect of hypoglycaemia on the output of both amines. This action of corticotrophin resembles the potentiation of the baroreceptor reflex that we have seen. There could thus be two parallel actions of corticosteroids, one on synthesis and the other on release. A further, and simpler, possibility, which seems to us to be compatible with all the work reviewed by Wurtman *et al.* (1972), is that induction of PNMT could be secondary to release of adrenaline, as happens with dopamine β-hydroxylase and other synthetic enzymes. If in fact steroids do not act directly on release, but only by the induction of synthesis, then it follows both from our results and from those of Wurtman *et al.* (1968) that enhanced synthesis and overflow of noradrenaline must be as important a part of the response as that of adrenaline.

In these experiments we have found that moderate hypoxia, with carotid arterial P_{O_2} between 6·0 and 6·5 kPa was sufficient to evoke the release of catecholomines. In previous experiments we found that a lower cartoid arterial P_{O_2} is needed to evoke the immediate, presumably neural, release. This suggests that the humoral mechanism may have a lower hypoxic threshold than the neural mechanism, and that it could thus have the greater physiological importance.

We are grateful for the expert assistance of Mr P. H. Whelpdale. P. Ellis is an M.R.C. Training Scholar.

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